"Extraction,characterizationandformulationofgelba sedpreparation fromliquorice(*Glycyrrhizaglabra*)plant".

A

PROJECT REPORT

BY

MS. SRUDHTI VILAS ANGRE

DEPARTMENT OF MICROBIOLOGY

VIVEKANAND COLLEGE, KOLHAPUR (AUTONOMOUS)

KOLHAPUR – 416003

2020-2021

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As a part of

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2020-2021

ROLL NO :- 8656

"Dissemination of education for Knowledge, Science and Culture"

-Shikshanmaharshi Dr. Bapuji Salunkhe

Shri Swami Vivekanand Shikshan Sanstha's

VIVEKANAND COLLGE, KOLHAPUR (AUTONOMOU)

Department of Microbiology

LABORATORY CERTIFICATE

This is to certify that **Ms. Srushti Vilas Angre** studying in B. Sc. III Microbiology at Vivekanand College, Kolhapur. She have sincerely completed Project Work entitled as "Extraction,characterization and formulation of gel based preparation **from liquorice (*Glycyrrhizaglabra*) plant"** prescribed by Vivekanand College, Kolhapur during academic year 2020-2021.

Mr. S. D. Gabale
Project Supervisor

Head of the Department

HEAD
DEPARTMENT OF MICROBIOLOGY
VIVEKANAND COLLEGE, KOLHAPUR
(AUTONOMOUS)

ACKNOWLEDGEMENTS

I wish to express my deep sense of appreciation to Prof. Mr. S. D. Gabale Department of Microbiology, Vivekanand College, Kolhapur for his valuable support and expert guidance during the course of this study. He has been extremely understanding and cooperative and has always taken great interest in this work.

I wish to express my sincere thanks to Head of the Microbiology Department Dr. S. P. Salokhe and Principal Dr. D. B. Patil, Vivekanand College, Kolhapur for providing the laboratory facilities in the Department to carry out the experimental work.

I express my thanks to my teachers, Ms. V. V. Misal and Ms. S. A. Pise for their valuable suggestions and help during the work. My special thanks to my teacher Mr. S. D Gabale for giving me the culture for study and his expert guidance throughout.

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Finally I thank my family members who had enclosed upon their blessing and moral and economical support because of which this work has proved satisfactory to me.

Place: Kolhapur

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Ms. Srushti Vilas Angre

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Place: Kolhapur

Date: **Ms. Srushti Vilas Angre**

Introduction:

Herbal medicine has its origins in ancient cultures. It involves the medicinal use of plants to treat disease and enhance general health and wellbeing. In fact, many pharmaceutical medications are based on man-made versions of naturally occurring compounds found in plants. Herbal medicines contain active ingredients. The active ingredients of many herbal preparations are as yet unknown. Some pharmaceutical medications are based on a single active ingredient derived from a plant source. Practitioners of herbal medicine believe that an active ingredient can lose its impact or become less safe if used in isolation from the rest of the plant. Herbal products are medicinal agents obtained from the plants. It's all started 1000 yearsagobyancientpeople.Sincesyntheticmedicinearenotyetinventedbythattime,ancient people had invented medicine out of the plants. Through generations the original herbal medicinehadbeenmodifiedduetothenewknowledgediscoveredandtechnologiesinvented. People often choose Herbal Medicine over the prescribed and conventional medicine when they have a lifelong health complication. More so, Herbal Medicine is not just to cure such health problems, it also improves the overall well-being, many health enthusiast use herbal medicine to preclude illness or to assure a healthier lifestyle.

The people are more attracting towards the use of herbal drugs to cure various types of diseases. For treatment of several diseases of human beings, plant drug 'Rasayana' hasalways played avital role. According to Worldhealth organization (WHO) morethan80%of the world populations are dependent on traditional medicine for their primary health care needs. Herbal medicine, also called botanical medicine or phytomedicine, refers to the use of plant parts such as seeds, berries, roots, leaves, bark, or flowers for medicinal purposes.Plants which are used for medicinal purposes contain various phytochemicals with beneficial biological activity such as antibacterial, antifungal, anticancer, antioxidant, antidiarrheal, analgesics and wound healing activity.

BriefHistoryofHerbalMedicine :

Herbal Medicineistheoldestformofhealingtothemankind.Herbalmedicinehadbeen used byalltheancientcivilizations–Chinese,Egyptian,Greek,Indian,Mesopotamianand

Roman. The first famous Herbalist was Hippocrates, known as the 'Father of Medicine' who stressed the importance of nature in healing .

HerbalMedicinecanbe broadlyclassified intothefollowingvariousbasic systems .

1. TraditionalChineseHerbalism,whichispartofTraditionalOriental Medicine.
2. Herbalism,whichisderivedfromAyurveda,and
3. Western Herbalism, which originally came from Greece and Rome to Europe andthen spread Ayurvedic to North and South America.

In the early 19th century, when methods of chemical analysis first became available,scientists began extracting and modifying the active ingredients from plants. Later, chemists began making theirown version ofplant compounds, beginning thetransition from rawherbs to synthetic pharmaceuticals. Over time, the use of herbal medicines declined in favor of pharmaceuticals.

Importanceof ofherbal medicine:

1. Playsinportatntroleinpreventionandtreatmentofvariousdisease.
2. People are greatly concerned about the efficacy and side effects of many synthetic drugs and hence choose herbal medicines for providing a safe and natural alternative treatment for many health problems.
3. .Herbalmedicines arenotaddictiveorhabit forming.
4. Herbal medicines combine with immune system of the human body to create an even detoxification process.
5. Itdoes nothaveany significantsideeffectcomparetosemisyntheticdrugs.

Limitationsofherbalmedicine:

1. Longerperiodoftimeisrequiredfortreatmentofdiseasesbyherbalmedicines than most prescription drugs.
2. optimizedinalaboratoryforeffectivenessand duetothis naturalness
3. Itcannothealappendicitisorheartattackseffectivelyasnodiagnostictestsor surgery is involved in it.

The liquorice root has a long tradition of medicinal use for the treatment of different types of diseases. Biologically active substances from the liquorice root exhibit the following effects: Antioxidant, expectorative, anti-inflammatory, anti-allergic, healing, spasmolytic, antiviral, antibacterial, and antiproliferative, etc. The dominant biologically active substances from Glycyrrhizae radix are glycyrrhizic acid (triterpene saponosides), chalcones (licurosid), and flavonoids(liquiritin,andliquiritigenin)[9-12].Therefore,thedevelopmentofdrugsfromthe liquoricerootwith antimicrobial activity forlocal used is an actual task.Theaim ofthis work was to carry out complex studies in the field of obtaining of a hydroalcoholic extract with antimicrobial activity from liquorice root for local use both, in medical and veterinary practice.

Reviewofliterature:

OriginOf*Glycyrrhizaglabra*:

The roots are unearthed in the autumn of the fourth season. It is grown in India, Spain, Iran, Russia, China and Italy.

Ecology-*Glycyrrhizaglabra*:

*Glycyrrhiza glabra*enjoys fertile, sandy, and clay soil near a river or stream where enough water is available for the plant to flourish in the wild, or under cultivation where it can be irrigated.

Morphology:*Glycyrrhizaglabra*

*Glycyrrhiza glabra*is herbaceous perennial, growing to 1 m in height, with pinnate leaves about 7–15 cm long, with 9–17 leaflets.

The flowers are 0.8–1.2cm long, purple to pale whitish blue, produced in a loose inflorescence.Thefruitisanoblongpod,2–3cmlong,containingseveralseeds.
The*Glycyrrhiza*shrub is a member of the pea family and grows in subtropical climates inrich soil. Below ground, the*Glycyrrhizaglabra* plant has an extensive root system with amain taproot and numerous runners. The main taproot, which is harvested for medicinal use, is soft, fibrous, and has a bright yellow interior.

BOTANICALDESCRIPTIONS:

*G.glabra*is a typical herbaceous perennial, growing to 1 m in height, presenting pinnate leaves with a length of 7 to 15cm. The flowers are purple to pale whitish blue, beingarrangedinahermaphroditeinflorescence,whereasthefruitisanoblonglegumewith2to 3 cm of length and containing several seeds.

The genus*Glycyrrhiza*(Fabaceae) consists of about 30 species,suchas*G.glabra*,*G. uralensis*,*G. inflata*,*G. aspera*,*G. korshinskyi*, or*G. eurycarpa*. Like the other plants of Fabaceae,*G.glabra*isabletofixnitrogen,duetosymbiosiswithbacteriaofthe

genus*Rhizobium*, at the root level, being suitable for sandy and clay soils, though preferring humid soils. Since the Egyptian age, the therapeutic properties of *G.glabra*are well documented). The roots are the most used parts whereas leaves are considered an agrochemical waste. However, in the last years, different authors studied the phytochemical composition of*G.glabra*leaves, demonstrating that certain compounds present in the roots are also identified in leaves, although in smaller quantities

G. aspera	*G. bucharica*
G. echinata	*G. eurycarpa*
G. glabra	*G. iconica*
G. inflate	*G. korshinskyi*

Phytochemicals:Compoundsfromtheplants:

Phytochemicals are defined as bioactive non-nutrient plant compounds in fruits, vegetables, grains, and other plant foods. They may provide desirable health benefits beyond basic nutrition to reduce the risk of chronic diseases. The prefix 'phyto' of phytochemicals is froma Greek word meaning plant. (https://shodhganga.inflibnet.ac.in/handle/10603/43972)

Various mechanisms of action of phytochemicals have been suggested. Phytochemicals exist in different forms such as antioxidants, hormonal action, stimulation of enzymes (stimulating certain enzymes may help reduce the risk of breast cancer), interfere with DNA replication (which would prevent multiplication of cancer cells), anti-bacterial properties, and lastly, by preventing physical adhesion of pathogens to cells in our body.

MainClassesofphytochemicals:

1 .Alkaloids :

Alkaloids are nitrogen-containing compounds widely distributed in various types of plant groups. Most are optically active. Alkaloids are classically defined as being plant derived, pharmacologically active, basic compounds derived from amino acids that contain one or moreheterocyclicnitrogenatoms.Alkaloidshavevariouspharmacologicalapplications.

Cocaine is a powerful nervous system stimulant. It is found in the leaves of Erythroxyloncoca .

Caffeine is the most widely consumed central nervous-system stimulant. Morphine exhibit narcotic effects; reserpine is an antihypertensive agent; atropine is a smooth muscle relaxant and strychnine is a nerve stimulant.

2. Flavonoids:

Flavonoids are a group of polyphenolic compounds with different chemical structure and Characteristics. They are widely distributed in the various plants. More than 4,000 different flavonoids have been identified within the major flavonoid classes which include anthocyanidins, catechins, chalcones, flavonols, flavones, flavanones, isoflavones, dihydroflavonols,etcInplants,Flavonoidsact asantioxidants,antimicrobials, photoreceptors, visual attractors, feeding repellants, and for light screening. It is also found that flavonoids exhibit various biological activities like antiallergenic, antiviral, anti-inflammatory, and vasodilating actions.

3..Glycosides:

Glycosides are defined as the condensation products of sugars (including polysaccharides) with a host of different varieties of organic hydroxy compounds .Salicin is an example of an alcoholic glycoside. Salicin is an antiinflammatory agent that is produced from willow bark. Digitoxin is a cardiac glycoside. It is used for treatment or prevention of cardiac arrhythmias. Barbaloin is found in Aloe vera. Barbaloin exihibit various pharmacological activity such as strong inhibitory effect on histamine release, anti-inflammatory, cathartic, antiviral, antimicrobial, anticancer, antioxidant activity and alternative for pharmaceutical or cosmetic applications.

4 .**Phenolics:**

Phenolics are widely distributed in the various plant flora and are the most abundant secondary metabolites of plants. phenolic compounds can be divided into three classes:

- 1.Shortly distributed (as simple phenols, pyrocatechol, hydroquinone, resorcinol, aldehydes derived from benzoic acids that are components of essential oils, such as vanillin),

- 2.Widelydistributed(dividedinflavonoidsandtheirderivatives,coumarinsand phenolic acids, such as benzoic and cinnamic acid and their derivatives) and
- Polymers(tanninandlignin)

Polyphenols are primarily recognized for their antioxidant activity and they also have other various biological activities like anti-inflammatory, anti-histamine, antibacterial and antiviral activities.

5.Saponins:

When saponin containing plants agitated in water, it forms soapy lather and so it's called saponin.

It is used also in beverages, food ingredients, shampoos, liquid detergents, toothpastes and extinguishers as an emulsifier and long-lasting foaming agent. Glycyrrhizin is a sweet- tasting triterpenesaponin which is isolated from the Glycyrrhiza plant and it is used as a medicine and as a sweetener and flavor enhancer in foods and cigarettes . Steroidal saponins are pharmaceutically very important due to their relationship to compounds such as the sex hormone, cortisone, diuretic steroids, vit.D and cardiac glycosides.

6 .Tannins:

Tannins have a protective function in the bark of the roots and stems, or any outer layers of plants. Because of their high polyphenol content, tannins areastringent in nature. Tannins are mainly divided into two categories: hydrolysable tannins and condensed tannins.

Hydrolysabletannins, uponhydrolysis,produce gallicacid and ellagic acid anddependingon the type of acid produced, the hydrolysable tannins are called gallotannins or egallitannins. Condensedtanninsaredimersoroligomersofcatechin,epicatechinorsimilarunits.Mixtures of these oligomers are powerful antioxidants known as oligomeric proanthocyanidins

Tanninsareused formedicinalpurposeduetotheirastringentproperties.Theypromoterapid healing and the formation of new tissues on wounds and inflamed mucosa .

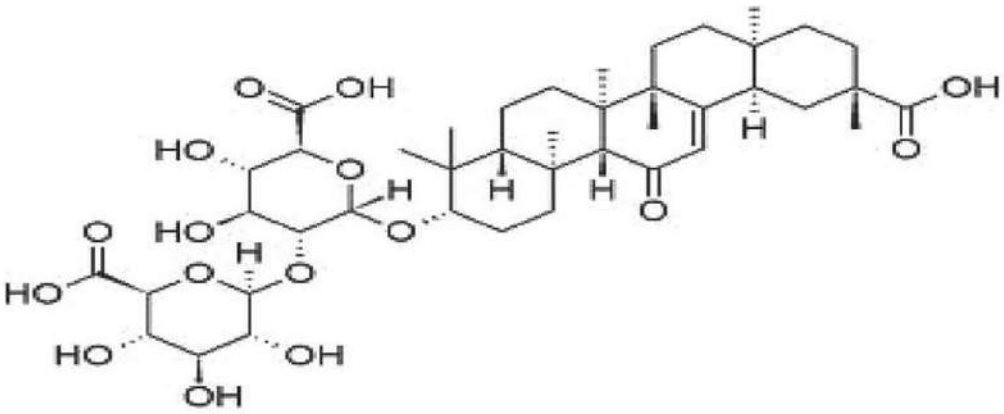
7 .Terpenoids:

Terpenes are a large class of natural hydocarbon secondary metabolites which consists of five-carbon isoprene units linked together most commonly in a head-to-tail arrangement.

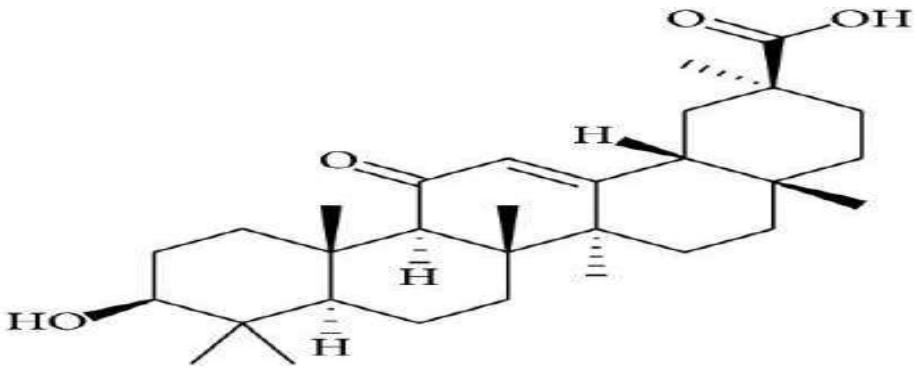
Terpenes are thus classified by the number of five-carbon units they contain : Hemiterpenes (C5), Monoterpenes (C10), Sesquiterpenes (C15), Diterpenes (C20), Sesterterpenes (C20), Triterpenes (C30), Carotenoids (C40).

Terpenoids exhibit a wide range of biological activities against cancer, inflammation,malaria, and a variety of infectious diseases. (Ali Esmail Al-Snafi, 2018)

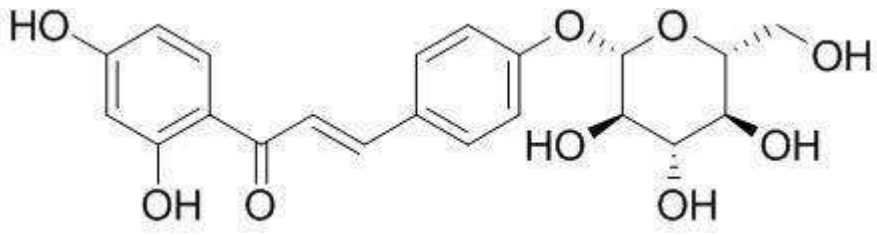
Structureofphytochemicalsof*glycyrrizaglabra*



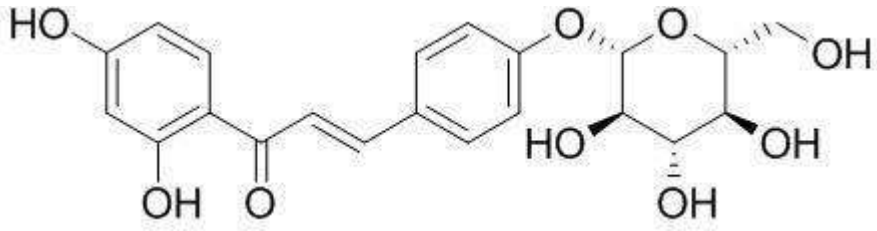
Glycyrrhizin



Glycyrrheticacid



Liquiritin



Isoliquiritin

Since the beginning of human cultivation practices, the role of plantsin medicine has been of huge importance. Glycyrrhiza glabra is one ofthe most popular medicinal plants belonging to the Fabaceae family(also known as Leguminosae), and its members are now commonlyused as feed and food. The genus Glycyrrhiza is derived from theGreek words glykos (sweet) and rhiza (root). It is also called licorice,liquorice, glycyrrhiza, sweet wood. Licorce commonly known as Yashthimadhu in Ayurveda is one such plant. Liquorice (Glycyrrhiiza glabra) symbolisesall that is wonderous in nature because, the it is used as traditional medicine for household remedy against various human ailmentsfrom antiquity. Liquoricewas supposed to have life-enhancing properties.

This species is a native of Mediterranean areas, but it is now also present in India, Russia, and China.The extracts are currently used in pharmaceutical and food industries, In the last fewdecadestherehasbeenanexponentialgrowthinthefieldofherbalmedicine.Itisgetting

popularized in developing and developed countries owing to its natural origin and lesser side effects.Plantderivateshadbeenemployedbypopulationtopreventdifferentkindofdiseases for centuries. The knowledge of plant properties was acquired by ancient civilization that passed down from generation to generation until today. Plant showed wide range of pharmacological activities including antimicrobial, antioxidant, anticancer, hypolipidemic, cardiovascular, central nervous, respiratory, immunological, antiinflammatory, analgesic antipyretic and many other pharmacological effects(Nicole Wittschier a, GerhardFaller,2009)

According to analysis of previous study which is done on licorice. Most of the study is reported on different part of licorice like root, stem and leaves. There is much study done on root, leaves than stem. Different part shows much similarities in phytochemical, antimicrobial and antioxidant properties. The phytochemical screening of the Glycyrrhiza glabra root revealed the presence of alkaloids, glycosides, carbohydrates, starches, phenolic compounds, flavonoids, proteins, pectin, mucilage, saponins, lipids, tannins, sterols and steroids. It showed memory enhancement, antidepressant, antimicrobial, anticancer, antioxidant, protective, anti-inflammatory, antiulcer, anti diabetic, hypolipidemic and many other pharmacological effects. It was used to have the functions of nourishing, alleviating pain, and relieving coughing. The canvas of the pharmacological activities when complied it stands out strongly as a drug of choice in various disorders. The main taproot, which is harvested for medical use is soft, fibrous, and has a bright yellow interior.

AntioxidantProperty:

Glycyrrhizin possesses the good antioxidant activity as it has the ability to scavenge the free radical wherever present in the blood circulation. (D Thakur, Abhilasha, 2016)Glycyrrhizin possesses the good antioxidantactivity as it has the ability toscavenge the free radical wherever present in the blood circulation. The radical scavenging activity may be defined as scavenging action of compound onto the free radical ions or reactive oxygen species (ROS) produced in human body. (D Thakur, Abhilasha,2016).Hydromethanolic root extract of G. glabra which consists of plenty of polyphenolic components can exhibit antioxidant activity and chemo preventive properties (D.M. Biondi, C. Rocco,2003)

AntimicrobialActivity:

Currently one of the major problems is multidrug resistant microorganism that spread rapidly and also the chronic conditions that caused by them. Glycyrrhiza Linn and its species are recognized to have selective antimicrobial activity due to isoprenoid phenols present in it.according to Varsha and Sonam (2013) this antimicrobial activity due to flavonoids, whereas Singh et al. (2015) reported that mostly isoflavones, Most recent studies have shown that there are significant antibacterial properties against gram positive and gram negative pathogens in hydromethanolic extracts of G. glabra. (H. Haraguchi, K. Tanimato, 1998)

Antibiofilm Activity :

Licorice compounds have also been suggested to be beneficial for dental caries and periodontal disease, through their anti-adhesion.glabridin and licochalcone A were found to exert antifungal activity towards C. albicans. (Celine Messier and Daniel Grenier, 2011). LicoriceextractevidencedinhibitorypotentialagainstthenineteentestedCandidastrains,but no pronounced effect was observed by testing the most abundant individual phenolic compounds. Candida tropicalis strains were the most sensible, followed by Candida glabrata, Candida parapsilosis and, then, Candida albicans.(Natalia Martins, Sonia Silva,2015)

Anti-inflammatoryactivity:

The anti-inflammatory activity of G. glabra and its use in the treatment of inflammatory diseases have been documented since ancient times (R. Yang, Yuan, Ma, Zhou, & Liu, 2017).It has the positive effects of G. glabra on the treatment of the upper respiratory tractand gastric system diseases. These pharmacological effects were due to an increase in the secretion of serotonin and prostaglandins in the stomach that led to a decrease of gastric inflammation (Bahmani et al., 2014).Furthermore, G. glabra is used in renal and liver complications on the basis of its strong anti- inflammatory effects (Y. Xiao et al., 2010). Y. Xiao et al. (2010) reported the inhibition of liver granuloma formation and the inflammatory cytokine production by glycyrrhizin, whereas X. R. Wang, Hao, and Chu (2017) describedthe anti-inflammatory effects on endometriosis. Moreover, Liu et al. (2017) proved the anti-inflammatory activity of glabridin on RAW cells

Antiulcerativeactivity:

The use of G. glabra extract as antiulcerative is widely known. For the gastrointestinal system, it is used in gastric and duodenal ulcers (Bardhan, Cumberland, Dixon, & Holdsworth, 1978), whereas for the treatment of spasmodic pains of chronic gastritis.The major compound responsible for this activity is glycyrrhizin, which can raise the concentration of prostaglandins in the digestive tract, promoting stomach mucus secretion (Jafarian&Ghazvini, 2007). In addition, liquorice prolongs the lifespan of stomach surface cells, demonstrating an antipepsin effect (Ram, Lachake, Kaushik, &Shreedhara, 2010).

Antiviralactivity:

The antiviral activity of G. glabra extracts against different viruses has been reported, including herpes simplex, Varicella zoster, Japanese encephalitis, influenza, and vesicular stomatitis virus (L. Wang, Yang, et al., 2015). It involved in the propagation of the cellular response to inflammation, activating T lymphocyte proliferation, and suppressing host cell apoptosis (L. Wang, Yang, et al., 2015). The antiviral mechanisms of both compounds are similar, inhibiting the adsorption and penetration of the virus in the early steps of the replicative cycle. Recently, the antiviral activity of glycyrrhizin against severe acute respiratory syndrome virus was evaluated (Cinatl et al., 2003). Glycyrrhizin affects the cellular signalling pathways such as protein kinase C, casein kinase II, and transcription factors, namely, activator protein 1 (Cinatl et al., 2003). Glycyrrhizin can also be used as a novel therapeutic method to control porcine epidemic diarrhoea virus (PEDV) infection, inhibiting the infection of Vero cells (namely, the entry and replication of PEDV) and decreasing the mRNA levels of proinflammatory cytokines (Huan et al., 2017).

Hepatoprotectiveactivity :

The hepatoprotective activity of glycyrrhizin and 18β-glycyrrhetic acid by inhibition of free-radical generation and lipid peroxidation has been extensively reported (Huo et al., 2011). According to Rizzato et al. (2017), glycyrrhizin and glycyrrhetinic acids prevent drug-induced liver injury and ensure the disruption of bile acid metabolism in humans.Indeed glycyrrhetinic acid has been reported as anti-inflammatory and hepatoprotective compound (Yin et al., 2017), whereas glycyrrhizin, when compared with the placebo,inducedasignificantreductionintheserumaminotransferasesandimprovedtheliver

histology (van Rossum et al., 1998). It has also been reported that the long-term use of glycyrrhizin prevents the development of hepatocellular carcinoma in chronic hepatitis C(van Rossum et al., 1998).

Anticarcinogenicandantimutagenicactivity:

Different studies suggest that the extract of G. glabra may be a potential supplemental source for different cancer treatments. C. S. Lee et al. (2008) demonstrated the toxic effect of G. glabra against the human cervix and uterus tumour cell line SiHa cells. Glycyrrhizin and glycyrrhetinic acids are effective compounds in gastric cancer treatment, whereasglycyrrhizin suppresses thromboxane A2 in lung cancer cell with low toxicity (Deng, Wang, Zeng, Chen, & Huang, 2017). According to S. Wang et al. (2017), 18β- glycyrrhetinic acid has antitumour activities in breast and ovarian cancer, gastric tumours, and leukaemia. Inliver cancer, the compound inhibits the proliferation of HepG2 cells without affecting the normal liver cell line. . Also, the anticancer activity in human leukaemia, by inducing the apoptosis of HL-60 cells through the activation of extrinsic and intrinsic apoptotic pathways, was proved by Y. C. Huang et al. (2016)

Neuroprotectiveactivity:

findings suggest that Liquorice stem extract has possible neuroprotective role of liquorice in the prevention of diseases such as Alzheimer. The basis of Alzheimer is the chronic inflammation of certain brain regions. Thus, the anti- inflammatory activity of liquoricemight contribute to the observed memory-enhancing effects (Yokota, Nishio, Kubota, & Mizoguchi,1998).Also,oxygenfreeradicalsareimplicatedintheprocessofagingandcould be responsible for the development of Alzheimer's disease in elderly persons. The protective role of liquorice extract may be attributed to its antioxidant properties, resulting in reduced brain damage and improvement of neuronal function and memory. The combination of anti-inflammatory and antioxidant activities with neuroprotective role could lead to memory-enhancing effects.

Sedativeactivity:

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervoussystem,beingGABAAreceptorsatarget foranaestheticsandneuroleptic,anxiolytic, andanticonvulsantcompounds(Simmleretal.,2013).G.glabraactsasamodulatorof

GABAA receptors (Hoffmann, Beltrán, Ziemba, Hatt, &Gisselmann, 2016), being able to induce sedative and anxiolytic effects.

Antidepressiveactivity:

Liquorice extract may have potential therapeutic value for the treatment of depressive disorders. Recent studies have shown that liquorice extract produces significantantidepressant effects in mice during forced swim test (FST) and tail suspension test (TST). The precise mechanisms by which liquorice extract produced this effect are not completely understood. However, it is suggested that the extract may interact with α1-adrenoceptors and dopamine D2 receptors, increasing the levels of norepinephrine and dopamine in the mice brain (Dhingra & Sharma, 2006).

Skineffects:

The main skin benefits reported for G. glabra are based on the antioxidant and anti-inflammatory activities as well as on the ultraviolet (UV)protection (Halder& Richards, 2004).fliquoricemainlyforskineruptions,includingdermatitis,eczema,pruritus,andcysts.
. Besides, glabrene acts as a tyrosinase inhibitor, preventing the formation of melanin in melanocytes, probably acting as skin-lightening agent. Saeedi et al. (2003) exposed that liquorice extract could be considered as an effective agent in the treatment of atopic dermatitis. Finally, the hydro-alcoholic extract of liquorice promotes hair growth, beingsafely used in herbal formulations for the treatment of various types of alopecia (Saumendu, Raj, Suvakanta, Jashabir, & Biswajit, 2014).

Objectives:

1. PhytochemicalextractionfromLiquoriceplant(stem)byusingvarious methods.
2. Purificationof phytochemicals.
3. Characterizationofextracted phytochemicals.
4. Antimicrobialactivityofliquoriceplant(stem)extractagainst*Pseudomonasaeruginosa* , *staphylococcus aureus.*
5. Growth curve of *Pseudomonas aeruginosa* , *staphylococcus aureus*aganist liquorice plant (stem) sample.
6. EffectofPH andTemperatureonliquoriceplant(stem)sample.
7. Stability study of formulated gel against*Pseudomonas aeruginosa* , *staphylococcusaureus.*
8. GC-MS analysis.
9. FTIRanalysis.

GLYCYRRHIZAGLABRA.

A. Materialsandmethods:

Plant sample:

Forstudies,weusedplantstemsamplepurchasedfromlocalAyurvedicstore.Stemsampleis further crushed by using grinder to make a fine powder .this powder is further used for extraction.

1. Preparationofplantextract:

a) **Methanolic extract** : Add 10gm (Bark &Cork) of liquorice stem extract and add in 100ml of methanol and put for extraction overnight on next day mixture is filtered with Whatman filterpaperno. 1, filtrate collected in flask used todetect antimicrobial activity.

b) **Ethanolic extract** : Add 10gm (Bark &Cork) of liquorice stem extract and add in 100ml of ethanol and put for extraction overnight on next day mixture is filtered with Whatman filter paper no. 1, filtrate collected in flask used to detect antimicrobial activity.

c) **Petroleum ether** : Add 10gm (Bark&Cork) of liquorice stem extract and add in 100ml Petroleum ether of and put for extraction overnight on next day mixture is filtered with Whatman filter paper no. 1, filtrate collected in flask used to detect antimicrobial activity.

d) **Chloroform** : Add 10gm (Bark&Cork) of liquorice stem extract and add in 100ml of chloroform and put for extraction overnight on next day mixture is filtered with Whatman filter paper no. 1, filtrate collected in flask used to detect antimicrobial activity.

e) **Sterilewater** : Add10gm (Bark&Cork)of liquoricestem extract and add in 100ml of sterile water and put for extraction overnight on next day mixture is filtered with Whatman filter paper no. 1, filtrate collected in flask used to detect antimicrobial activity.

FIG.Preparationofplant(stem)extract:

2. Phytochemicaltestsofplantextract:

a) Testforalkaloids :

3 ml of extract was evaporated to dryness and residue was heated on boiling water bath with 2N HCL (5 ml) after cooling the mixture was filtered and the filtrate was divided into two equal portions. One portion was treatedwith thefewdrops ofMayer's Reagent and other with equal amount of Warner's Reagent). The sample was then observed for the presence of turbidity or precipitation. '+' score was recorded if the reagent indicated the presence of compound. '-' score was recorded for absence of compound.

b) Testforflavonoids :

5 ml of each extract was treated with few drops of concentrated 2N HCL and Magnesium turnings (0.5 gm). Presence of flavonoids was indicated if pink or magenta colour developed within 3 min.

c) TestforGlycosides:

Small amount of alcoholic extract of sample dissolved in 1ml water & then addition of aq. Sodium hydroxide, yellow colour indicates the presense of glycosides.

d) Testforsteroids[salkowski'stest]:

preparetheextractwith2gmofplantpowder and50mlchloroformandEvaporateit,thenadd chloroform same flask and add sulfuric acid observe for lower layer reddish brown colourand at interface the presence of steroid ring.

e) Testforcardiacglycosides[kellerkilliani'stest]:

100mg ofextract dissolved in 1ml ofglacial aceticacid containing onedrop offerricchloride solution & 1ml of concentrated sulfuric acid added. A brown ring observed at the interface indicates the presence of a deoxy sugar characteristic of cardenolides.

f) TestforResins :

2ml of chloroform or ethanolic extract 5 to 10 ml acetic anhydrite added & dissolved by gently heating. Aftercooling 0.5 ml ofH_2SO_4 was added, Bright purplecolourwas produced; it indicates the presence of resins.

g) Testforphenols[ferricchloridetest]:

1ml of alcoholic solution of sample, 2ml of D/W followed by a few drops of 10% aq. Ferric chloride solution was added. Formation of blue or green color indicated the presence of phenols.

h) Tannintest:

Atesttubecontainingabout5mlofanaqueousextractafewdropsof1%solutionoflead acetate was added. Formation of a yellow or red precipitate indicated the presence of tannins.

i) TestforTerpenoids:

2ml of chloroform & 1ml of conc.H_2SO_4 was added to 1mg of extract &observed for reddish brown color that indiacted the presence of terpenoid.

j) Testforsaponin:

About 2.5 gm of dried sample powder was extracted with boiling water. After cooling the extract was shaken vigorously to froth and then allowed to stand for 15 to 20 min. and classified for soponin content as follows. No froth = negative; froth less than 1 cm = weakly positive; froth 1.2 cm high = positive; and froth greater than 2 cm high= strongly positive

k) Testforcoumarin :

3mlof10%NaOHwasaddedto2mlofaqueosusextractformationofyellowcolour indicates coumarin.

l) Amino acid:

ninhydrintest:tothe2mlexract2mlonninhydrin reagentwasaddedtoboilforfewminutes, formation of blue colour indicates the presence of amino acid.

m) Diterpenes:

copper acetatetest: extract weredissolvedin waterand treated with 10 drops ofcopper acetat solution, formation of emerald green colour indicates presence of diterpenes.

n) Leucoanthocyanin:

5ml isoamylalcohol added to 5ml of aqueous extract, upper layer apper red in color indicates the presence of Leuanthocyanin.

3. Antimicrobialstudyofpreparedphytochemicalextract:

The antibacterial activity of the various organic solvents extracts such as chloroform, methanol, petroleum ether, ethyl acetate, ethanol and aqueous extract was carried out by agar cup method. From each extract ethanolic extracts were choosen for further studies. The reference microorganisms for antibacterial activity testing were used as *pseudomonas aeroginosa , staphylococcus aureus* for present study. From ethanolic solvent extract 100 µl of sample was added in a well and their respective solvent as control. Plates were kept for diffusion of sample in refrigerator for 10 min and then incubated at 37°C for 24 hours. After incubation period plates were observed for zone of inhibition.

4. PurificationofplantextractbyusingColumnChromatography :

Activation ofcolumn :

50gm of silica gel (with mesh size- 60 to 120) washed 2 times with ethanol. Dried in oven overnight. Set column chromatography,3ml of plant extract sample was added. run the sample. Collect 3ml fractions in 60 skrew cork test tubes. Evaporate fractions at 60degree centigrade in oven. Check the fractions for antimicrobial activity.

FIG. Column chromatography

Antimicrobialactivity:

Antimicrobial activity of collected fractions were done by disc diffusion method. Paper disc deep inside respective fractions for overnight. Next day disc is kept on media Mueller Hinton for *pseudomonas aeruginosa* and *staphylococcus aureus*. plates were kept for 24hrs incubation. And check for zone of inhibition.

5. TLCanalysis:

TheTLCanalysiswerecarriedoutfortheliquoricesteamextract.Themodifiedmobile system is used like ethyl acetate: toluene: methanol in 1:8:1 for analysis.

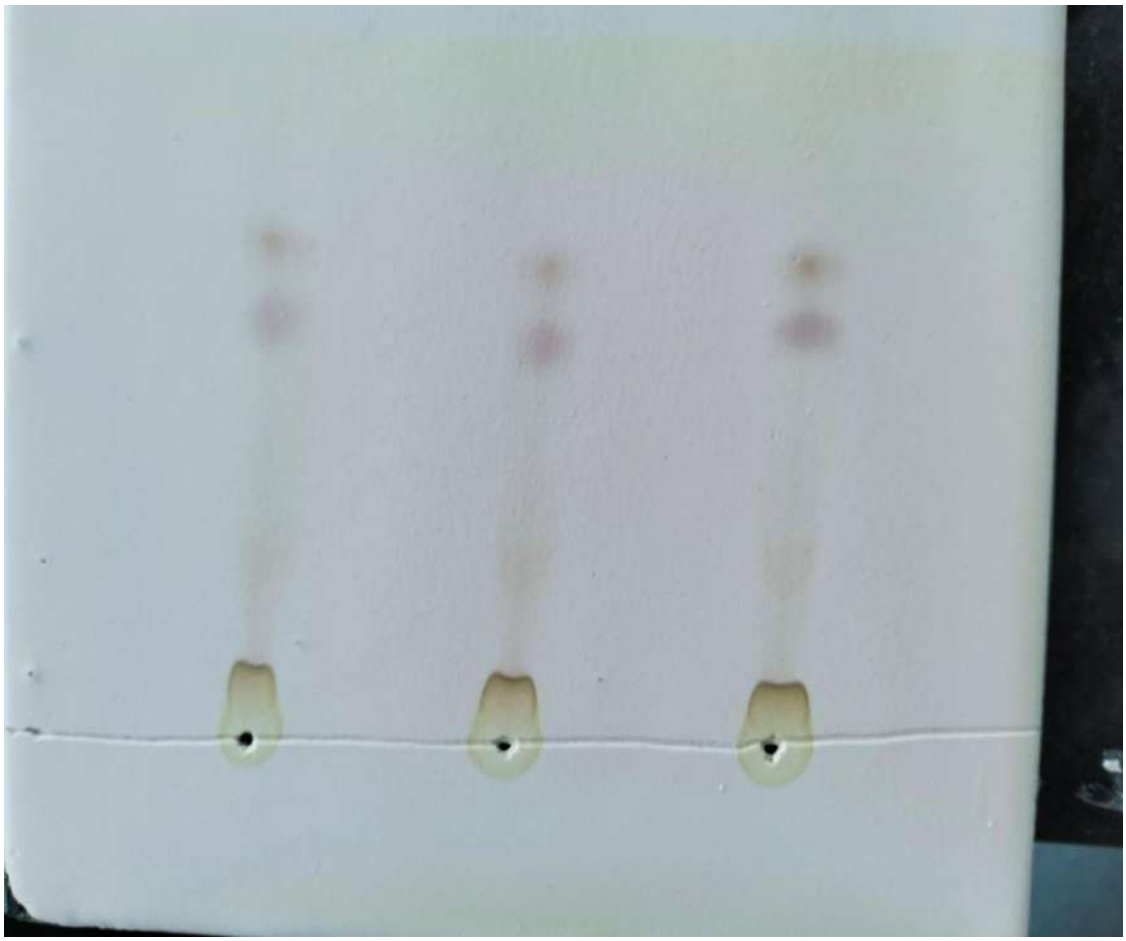


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FIG. Thinlayer chromatography

Antimicrobialactivity :

Antimicrobial activity of separated bands were done by well diffusion method. All three bands were scratched by using capillary and collected in ethanol as a solvent. The tubes were kept for settling overnight and the sample was used for further studies. . From collected tubes 100 µl of sample was added in a well. Plates were kept for diffusion of sample in refrigerator for 10 min and then incubated at 37°C for 24 hours. After incubation period plates were observed for zone of inhibition.

6. EffectofPHandTemperatureonliquoriceplant(stem)extract:

To check the effect of various pH and temperature on liquorice plant (stem) extract. Same experiments was reapeated atvarious pHthat is from4 to 9.Muller Hinton agar was prepared in 6 different flask andadjustthe pH by pH meter.inoculate the 100µl sample in the well.kept all the plates for diffusion for 10 min.afterthatkept all the plates in incubator at 37°c for24hrs.and observe the zone of inhibition.

Experiment was carried out at various temperature20 to 70°c. Muller Hinton agarplateswasprepared.inoculatethe100µlsampleinthewell.keptalltheplatesfordiffusionfor 10 min. Afterthat each plate is placed at various temperature like refrigerator,room temperature,incubator and oven for 24hrs and observe the zone of inhibition.

FIG.EffectofpH

7. Stabilitystudyofformulatedgel:

Preparation of liquorice gel using aloevera. The aloevera leaf washesd with sterile water and outer layer was removed by using knife. The knife was introduced in mucilage layer. Byusing spatula the pulp was collected in a beaker. The extracted gel wasfiltered by muslin cloth. Different dilutionsofplant extract and aloeveragel werepreprepared. Dilutions like7:3, 8:2 ,9:1. Then extracted gel was used for further studies.

Antimicrobialactivityofformulatedgel:

The Muller Hinton agar plates were prepared. inoculateeach dilutions of 100µl sample in 3 diiferent wells.kept the plates for diffusion for 10 min afterthatkept all the plates in incubator at 37°c for24hrs.and observe the zone of inhibition. The stability study was checkedfor3monthsattheintervalof15days.Forstabilitystudy *pseudomonasaeroginosa, staphylococcus aureus*organisms used.

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8. Extractionofliquoriceoil:

Soxhlet apparatus is used for extraction of oil. Petroleum ether is used as solvent for this method. Exactly 10gm of liquorice stem powder is weighed and sealed in Cellulose thimbles and packed with Whatmann filter paper.After completion of 12 cycles stop the process and collect the extracted oil with petroleum ether and kept for evaporation at 60°c for 20 min. Then the remaining extracted oil was collected.

FIG : Soxhlet apparatus.

9. Gaschromatographymassspectroscopy(GC-MS):

The Gas Chromatography/Mass Spectrometry (GC/MS) instrument separates chemical mixtures (the GC component) and identifies the components at a molecular level (the MS component). It is one of the most accurate tools for analysing environmental samples. TheGC works on the principle that a mixture will separate into individual substances when heated. The heated gases are carried through a column with an inert gas (such as helium). As the separated substances emerge from the column opening, they flow into the MS. Mass spectrometry identifies compounds by the mass of the analyte molecule. A library of known mass spectra, covering several thousand compounds, is stored on a computer. Mass spectrometry is considered the only definitive analytical detector.

Ethanolic plant stem extract was analysed by GC–MS. By using a mass spectrophotometer QP 5000 (Shimadzu). Theionization voltagewas set at 70 eV and chromatography of sample was done by temperature programming method with a Resteck column having 0.25 mm × 30 mm;XTI-5dimensions.The initial temperature of column was set at 40°c for 4 min and then increased upto 300°c linearly with 11°c min−1 rate and held for 29 min. The temperature of injection port was set at 275°c and GC–MS interface was maintained at 300°c and heliumwas used as carrier and the flow rate of gas was adjusted at 1 ml min−1 and48 min run time.

10. Fouriertransforminfraredspectroscopy(FTIR):

when infrared radiation passes through a sample , some of the radiation is absorbed. The radiation that passes through the sample is recorded. The frequency range is measured as4000 Itis themost common formofinfrared spectroscopy. It isbased ontheprinciplethat “ - 600 cm-1.

In short, the IR spectrum is divided into three wavenumber regions: far-IR spectrum (<400 cm-1), mid-IR spectrum (400-4000 cm-1), and near-IR spectrum (4000-13000 cm-1). The mid-IR spectrum is the most widely used in the sample analysis, but far- and near-IR spectrum also contribute in providing information about the samples analyzed.

Themid-IRspectrumisdividedintofourregions:

(i) thesinglebondregion(2500-4000cm-1),

(ii) thetriplebondregion(2000-2500cm-),

(iii) thedoublebondregion(1500-2000cm-1), and

(iv) thefingerprintregion(600-1500cm-1).

11. Microbialgrowthcurveanalysis:

For monitoring the microbial growth curve kinetics, the test organisms *pseudomonas aeroginosa , staphylococcus aureus* used. The ethanolic extract of 5ml was added to the Muller Hinton broth in the one side arm flask with 1 ml suspension of 18hr old culture of *pseudomonas aeroginosa , staphylococcus aureus.* The second flask was used as a control with media and extract in it. For obtaining the standard growth of organisms the third flask was used which contained Muller Hinton broth and 1 ml of suspension. The fourth flask containing only the media was used as a control. During 18hrs incubation , the absorbance (620nm)measurementswereperformedeveryhourandtheregularorbitalshakingat40°cat
140 rpm. The optical density was measured by using colorimeter at 620nm for both organisms.

B. ResultandDiscussion:

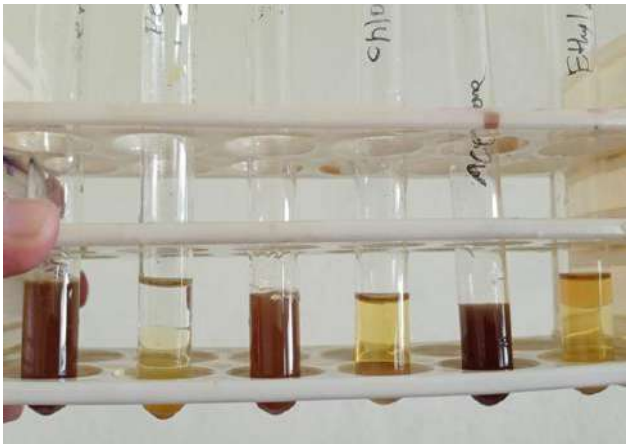
2.PhytochemicalAnalysis:

Liquorice is one of the most widely used and extensively researched medicinal plants of the world, A large number of biological active compounds have been isolated from *Glycyrrhiza species* .Hence we used extract of stem for the study of phytochemical extract in various solvents . It was observed that extraction of stem bark and stem cortex by using different solventsuchasEthanol,Methanol,EthylAcetate,PetroleumEther,ChloroformandSterile Water in below table .

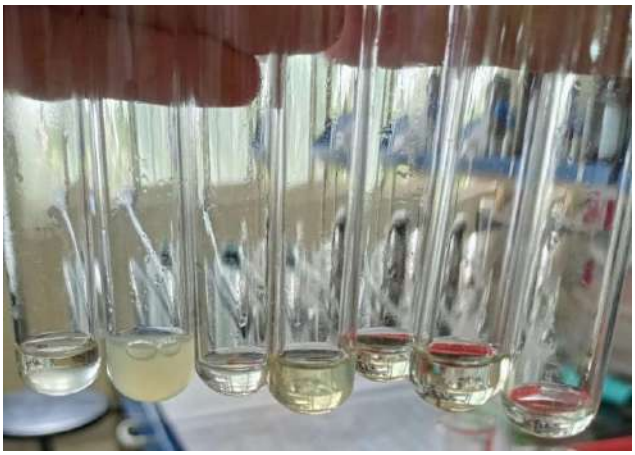
Table1.Qualitative Analysis of Phytochemical in various solvents (Liquorice plant stem).

	Ethanol	**Methanol**	**Ethyl Acetate**	**Petrolium Ether**	**Chloroform**	**Aqueous**
Alkaloids	+	+	+	+	+	+
Flavonids	-	-	-	-	-	-
Glycosides	-	+	-	+	-	+
Phenol	-	-	-	-	-	-
Tanins	-	+	-	-	-	-
Terpenoids	+	-	-	-	-	-
Cardiac glycosides	+	-	-	+	+	-
Steroids	+	-	-	-	+	-
Resins	+	+	-	-	-	+
Coumarin	-	+	+	+	+	+
Leucoanthocyanin	-	-	-	-	-	-

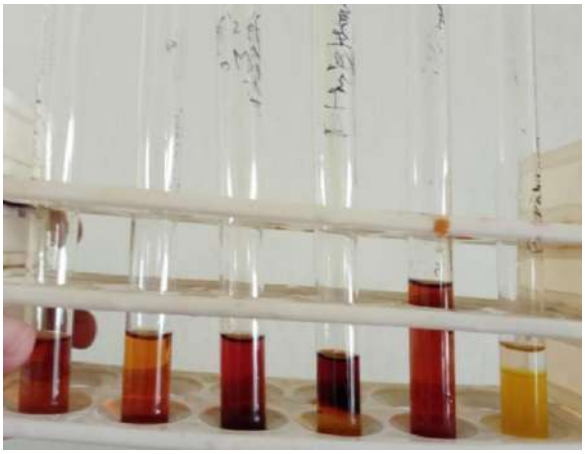
Note:'+'indicatespresenceand '-'indicates absenceofcompounds.



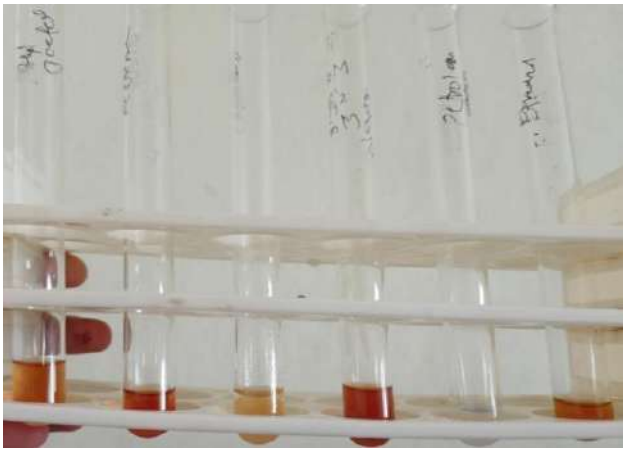
Testfor phenol



Testfor alkaloids



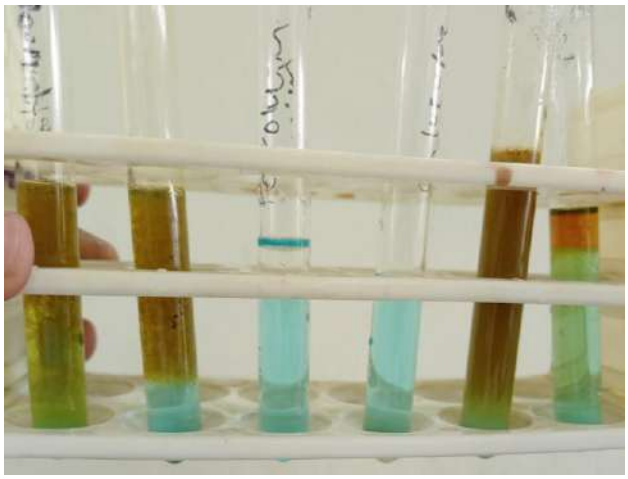
Testforcardiac



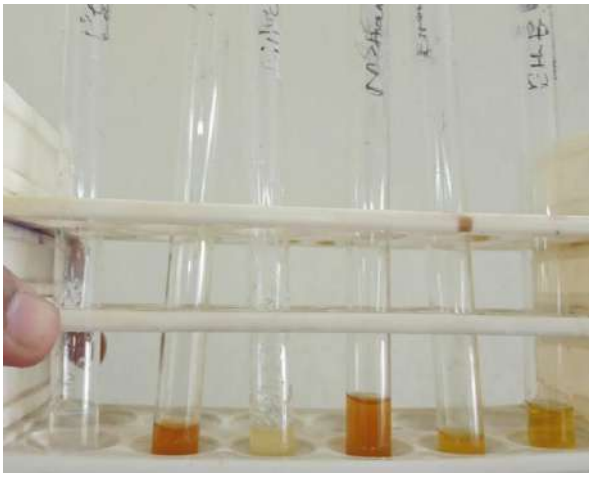
Testfor flavonoids

Testfor terpenoids

Testfor diterpins



Testfor resins



Testfor tanins

(Phytochemical test by using different solvent extract forStem sample of plant Liquorice.)

3. Antimicrobialactivityofphytochemicalextract:

The antimicrobial activity of extract was studied by the presence of inhibitory zone. The antimicrobial activity was studied by using extract against different micro-organim such as *staphylococcus aureus* and *Pseudomonas aeruginosa.*Amongst all extract ethanol extract shows good antimicrobial activity.

stap

hylococcusaureus

Pseudomonas aeruginosa

Tableno-2.Antimicrobialactivityofplant(stem)extract:

Sr.no	**Organisms**	**Zoneofinhibition(mm)**
1	*staphylococcusaureus*	12mm
2	*Pseudomonasaeruginosa*	-

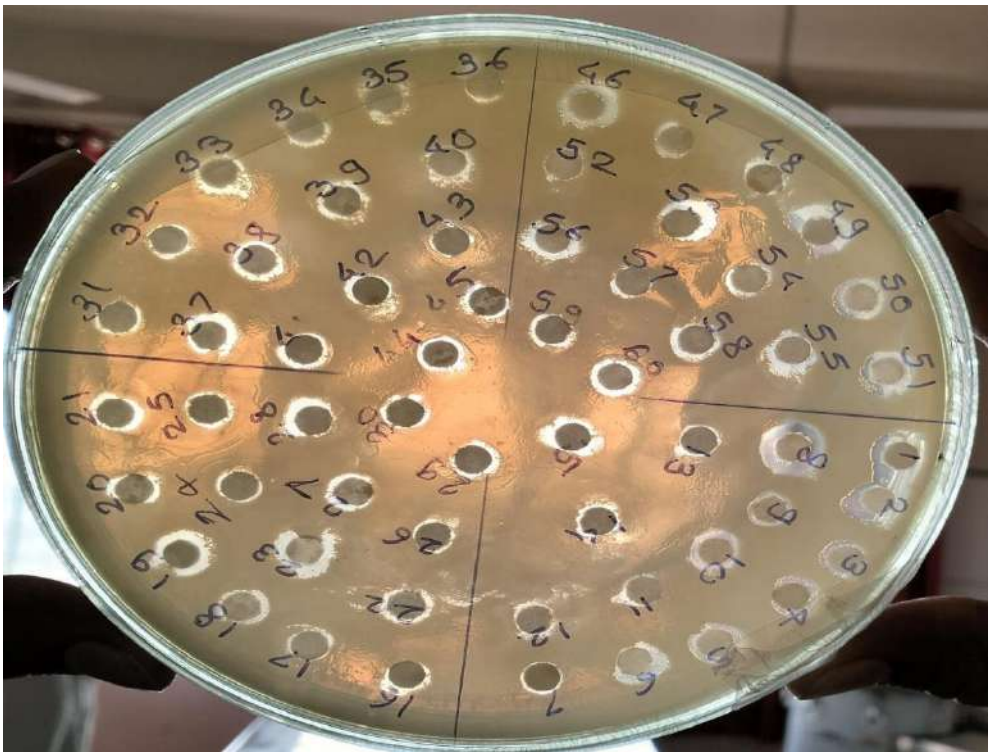
The liquorice plant (stem)extract was sensitive to the *staphylococcus aureus*and resistant to the *Pseudomonas aeruginosa.*

4.SeparationofCompoundbyusingcolumnChromatography:

The ethanol extract was used for separation of compound form these 60 fractions were separate out. The antimicrobial activity of these 60 fractions were checked out aganist *Pseudomonas aeruginosa and staphylococcus aureus.* Among these 60 fractions,fraction number45 , 58of *Pseudomonas aeruginosa* shows large inhibitory zone. Fraction number 22 , 54 of *staphylococcus aureus*shows large inhibitory zone . These fraction used forfurther studies.

)Antimicrobialactivitydonebydiscdiffusionmethod-

A. *Pseudomonasaeruginosa* -



B. ***staphylococcusaureus.-***

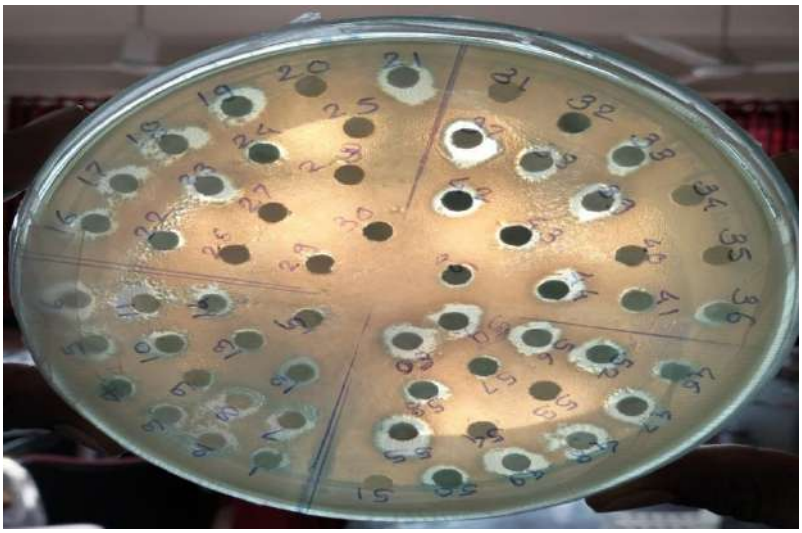


Table.3–Antimicrobialactivityofcollectedfraction:

	Organisms	**Fractionnumber**	**Zoneofinhibition. (mm)**
1.	*Pseudomonas aeruginosa*	45	8
		58	10
2.	*staphylococcusaureus.*	22	7
		54	6

EffectofPHonliquoriceplant(stem)extract:

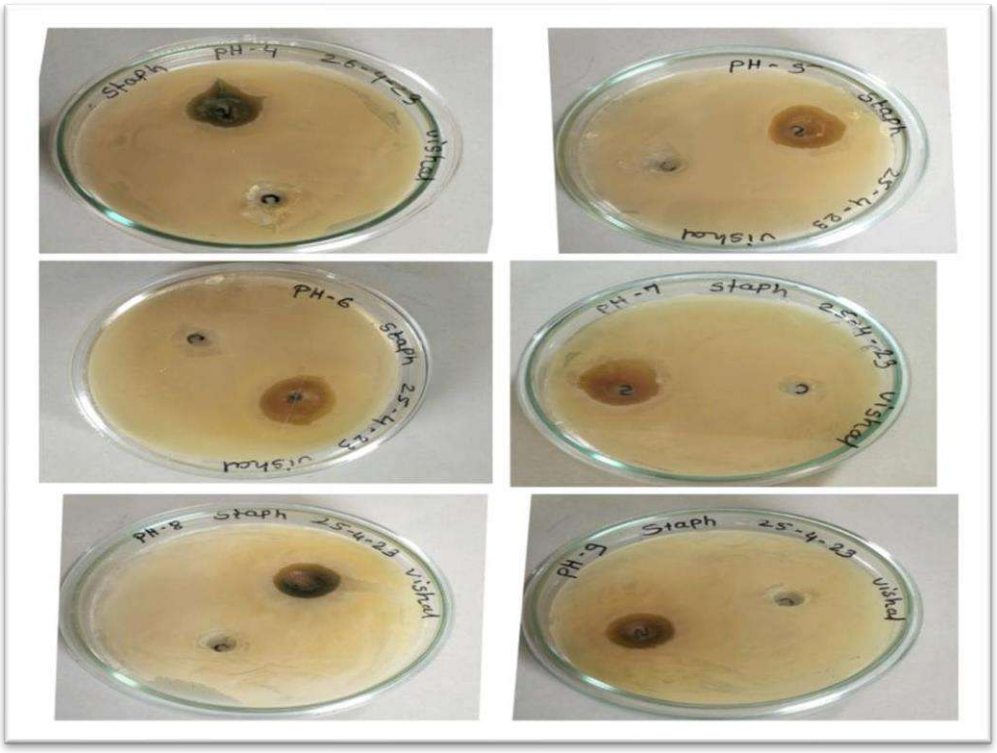


FIG.EffectofpHon plant extract against*staphylococcus aureus*

TheeffectofpHonplantextractwhichshowszoneofinhibitionagainst*staphylococcus aureus* given as follows :-

Table– 4.

PH	**Zoneofinhibition (mm)**
4	17
5	14
6	13
7	15
8	10
9	9

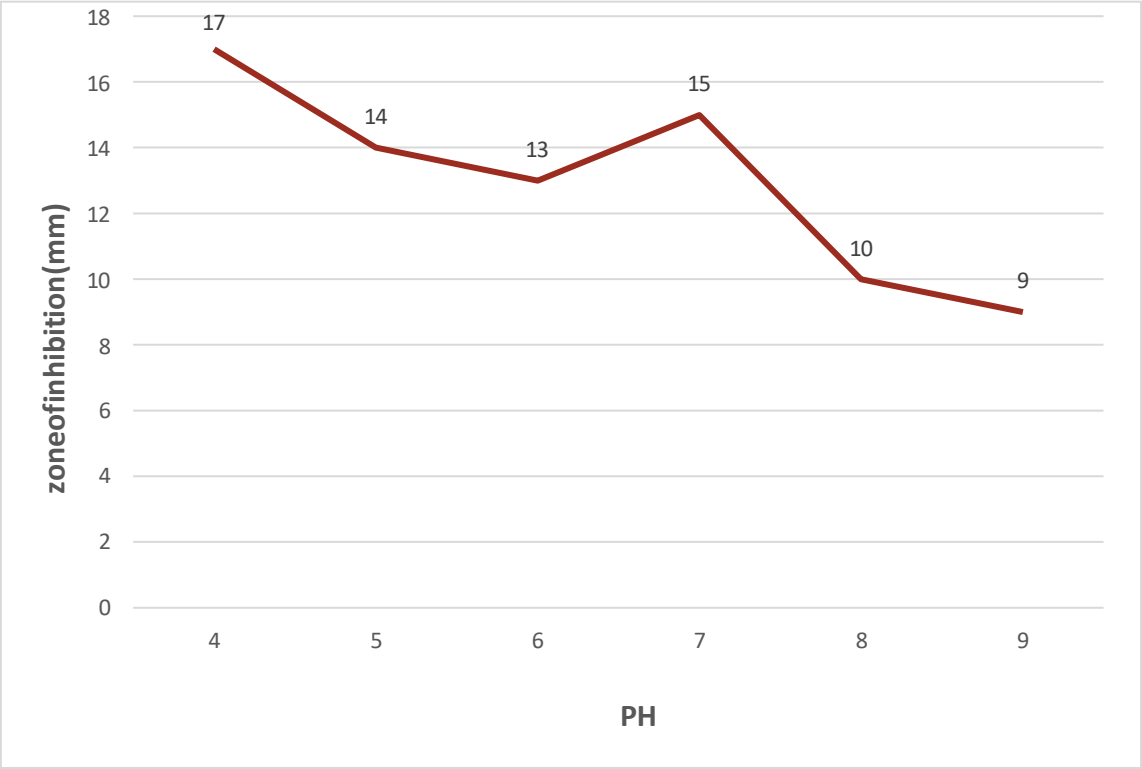


FIG-ZoneofinhibitionvsEffectof PH

EffectofTemperatureonliquoriceplant(stem)extract:

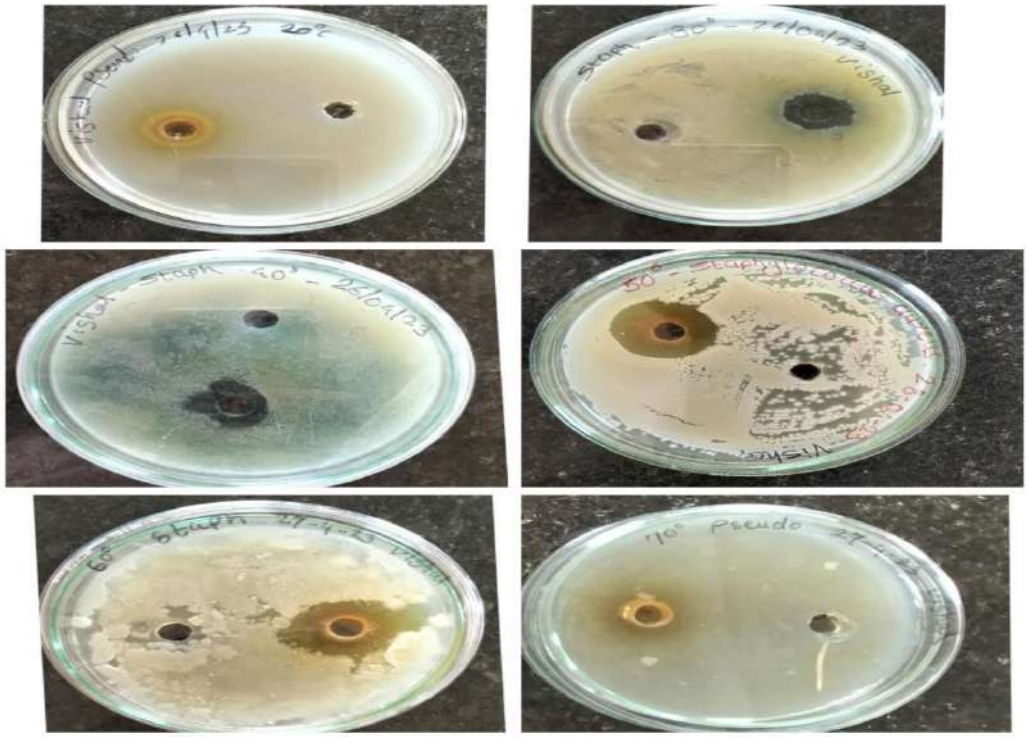


FIG.EffectofTemperatureon plantextractagainst*staphylococcus aureus*

Theeffectoftemperatureonplantextractwhichshowszoneofinhibitionagainst *staphylococcusaureus*givenasfollows :-

Table –5.

Temperature (°c)	**Zoneofinhibition (mm)**
20°	19
30°	12
40°	11
50°	17
60°	15
70°	0

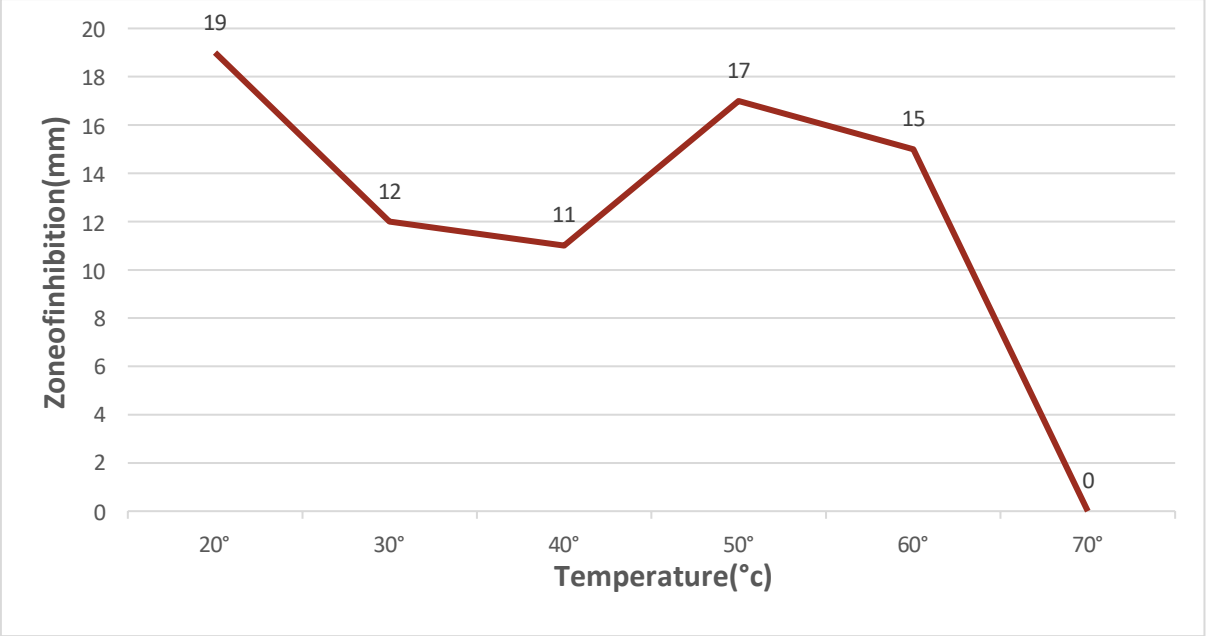


FIG -Zoneof inhibitionvsEffectofTemperature.

Antimicrobialactivityofseparatedbands:

The3bandswereobservedonTLCplate.3bandswerenamedasupperband1,2,3.Their zone of inhibition as follows :-

FIG.Antimicrobialactivityofseparatedbands:

Table 6–

organism	**Zoneofinhibition(mm)**		
	I	**II**	**III**
staphylococcusaureus	4	5	3

Antimicrobialactivityofformulatedgel:

Date:10/3/23. Date:25/3/23

FIG:Antimicrobialactivityofformulatedgel:

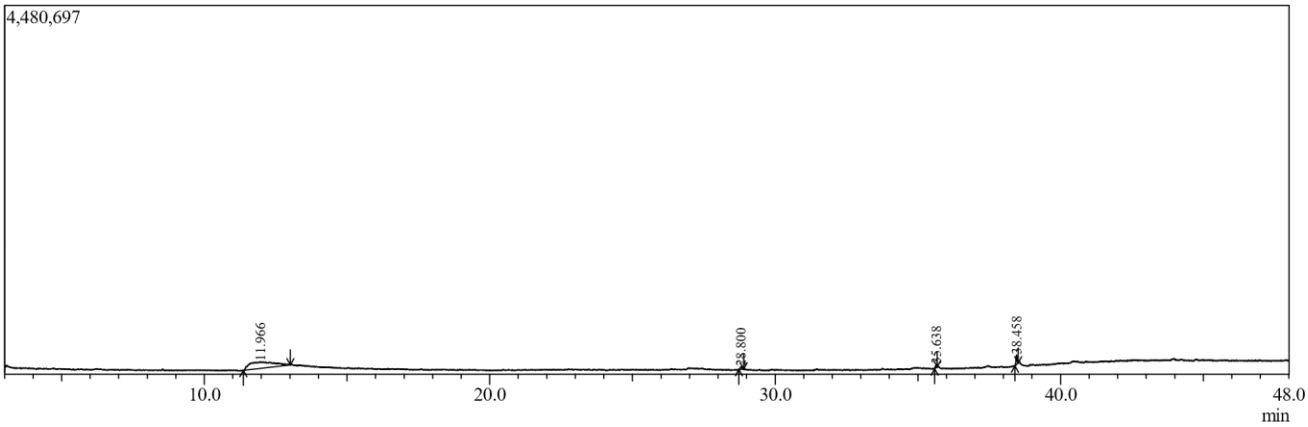
Table 7 :-

organism	**Zoneofinhibition(mm)**			
	Date	**7:3**	**8:2**	**9:1**
staphylococcus aureus	10/3/23	6	6	5
	25/3/23	8	7	7
	9/4/23	9	10	9
	24/4/23	7	8	9

Thestability studyof formulatedgel was checked for2 months by performingantimicrobial activity against *staphylococcus aureus.*

8. Gaschromatography-Massspectrometryanalysisofextractedoil:

Oil was used for GCMS. The oil was extractedby using **Soxhlet apparatus.** Further GCMS profiling of the extract together revealed the occurance of a total 4 major peaks were identified. These compound belong to different chemical classes and most of them are reported to exhibit important biological activities The identified compounds with their peak number, retention time (RT) and peak area (%) are presented in table.



PeakReportTIC

Peak#R.TimeI.TimeF.TimeAreaArea%Name

1 11.966 11.365 13.015
4754974 88.462-Pyrrolidinone,1-methyl-

2 28.800 28.725 28.895
144480 2.692,6,10-Trimethyltridecane

3 35.63835.58535.6801171792.18
Hexadecanoic acid, ethyl ester

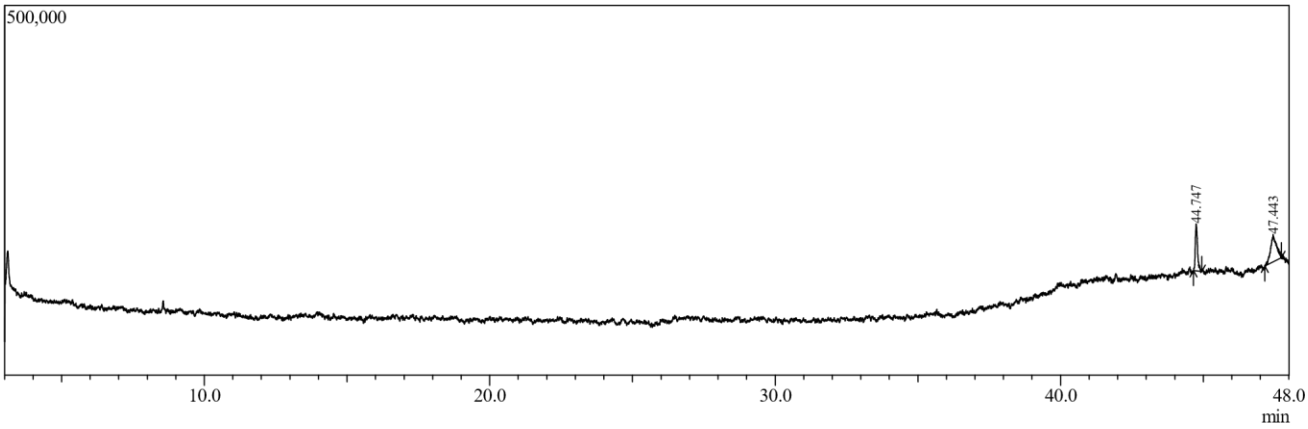
4. 38.45838.40538.5153588596.68
Butyl9,12-octadecadienoate

Table.8 :-

Nameof compound	**Biological significance**
2,6,10-Trimethyltridecane	Plantmetaboliteand volatileoil component.
Hexadecanoicacid,ethylester	Usedas ahair andskin conditioning agent.

9. Gaschromatography-Massspectrometryanalysisofcollectedfractions:

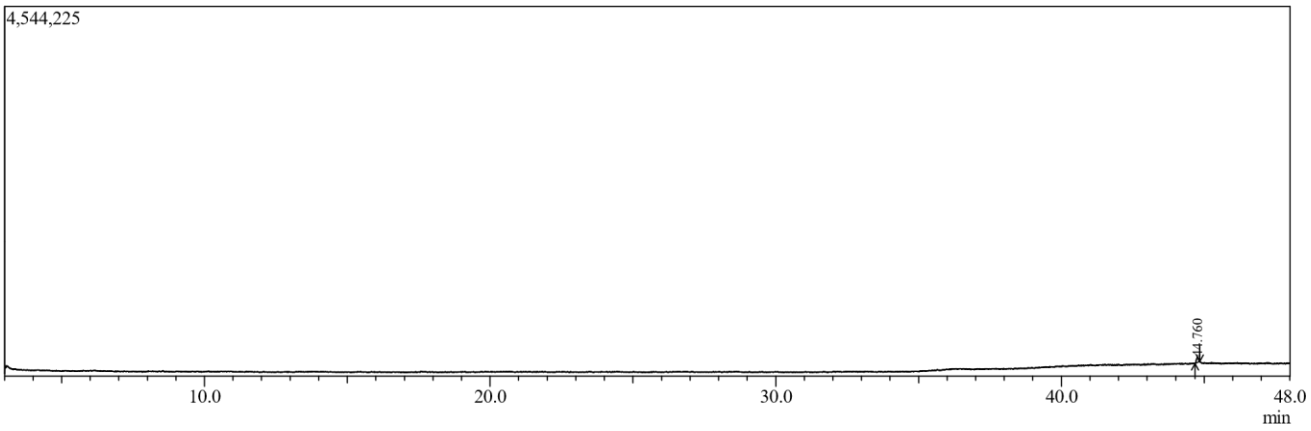
Fraction 22



PeakReportTIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Name
1	44.747	44.660	44.945	362116	39.57	Bis(2-ethylhexyl) phthalate
2	47.443	47.150	47.735	553061	60.43	.beta.-Tocopherol, O-methyl-

Fraction 45 :

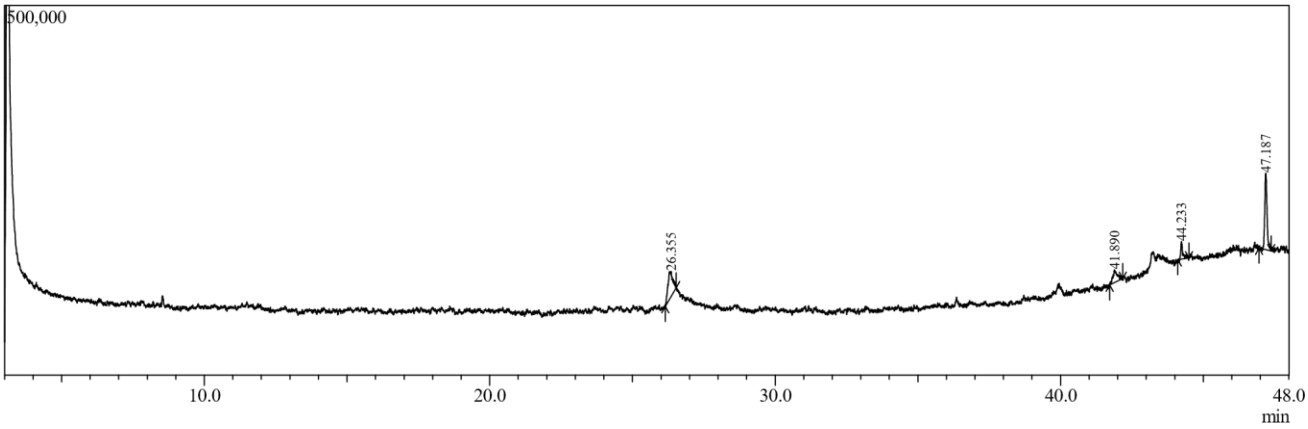


PeakReportTIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Name

1	44.760	44.680	44.850	323597	100.00 Bis(2-ethylhexyl) phthalate

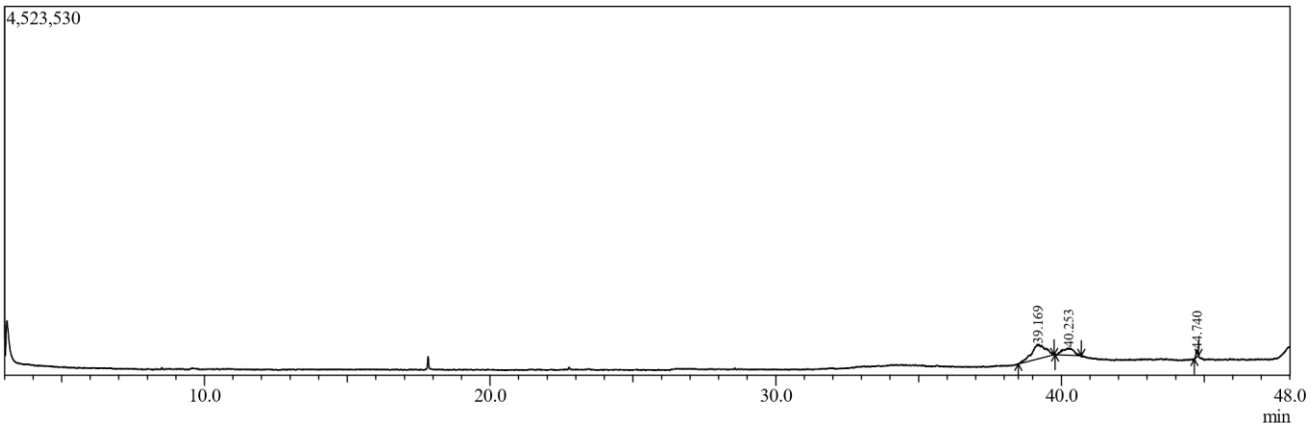
Fraction54:



PeakReportTIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Name
1	26.355	26.155	26.535	46956133.50		Diethyl Phthalate
2	41.890	41.720	42.180	20327814.50		.alpha.-Tocospiro B
3	44.233	44.100	44.495	115057	8.21	Hexanedioicacid,bis(2-ethylhexyl) ester
4	47.187	46.950	47.375	61379643.79		Bis(2-ethylhexyl) phthalate

Fraction58:



PeakReportTIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Name
1	39.169	38.485	39.750	5999379	68.75	.alpha.-Amyrin
2	40.253	39.780	40.700	2372917	27.19	Betulinaldehyde

3	44.740	44.650	44.800	354212	4.06	Bis(2-ethylhexyl) phthalate

Table no 9.

Nameof compound	**Biological significance**
.beta.-Tocopherol,O-methyl-	Antioxidant activity and increases oil stability.
Bis(2-ethylhexyl)phthalate	Apoptosis inhibitor,involved in cell proliferation.
DiethylPhthalate	Teratogenic agent, a neurotoxin and a plasticiser.
alpha.-TocospiroB	Anti-inflammatoryactivity.
Hexanedioicacid,bis(2-ethylhexyl)ester	Used in production of plastics and pharmaceuticals.
.alpha.-Amyrin	It has anti-infiammatory,anti-nociceptive,gastroprotective and hepatoprotective properties.
Betulinaldehyde	Effectiveforadjuvanttherapytotreatlung cancer.

10. Fouriertransforminfraredspectroscopy(FTIR)analysisofcollected fractions :

Fraction 22

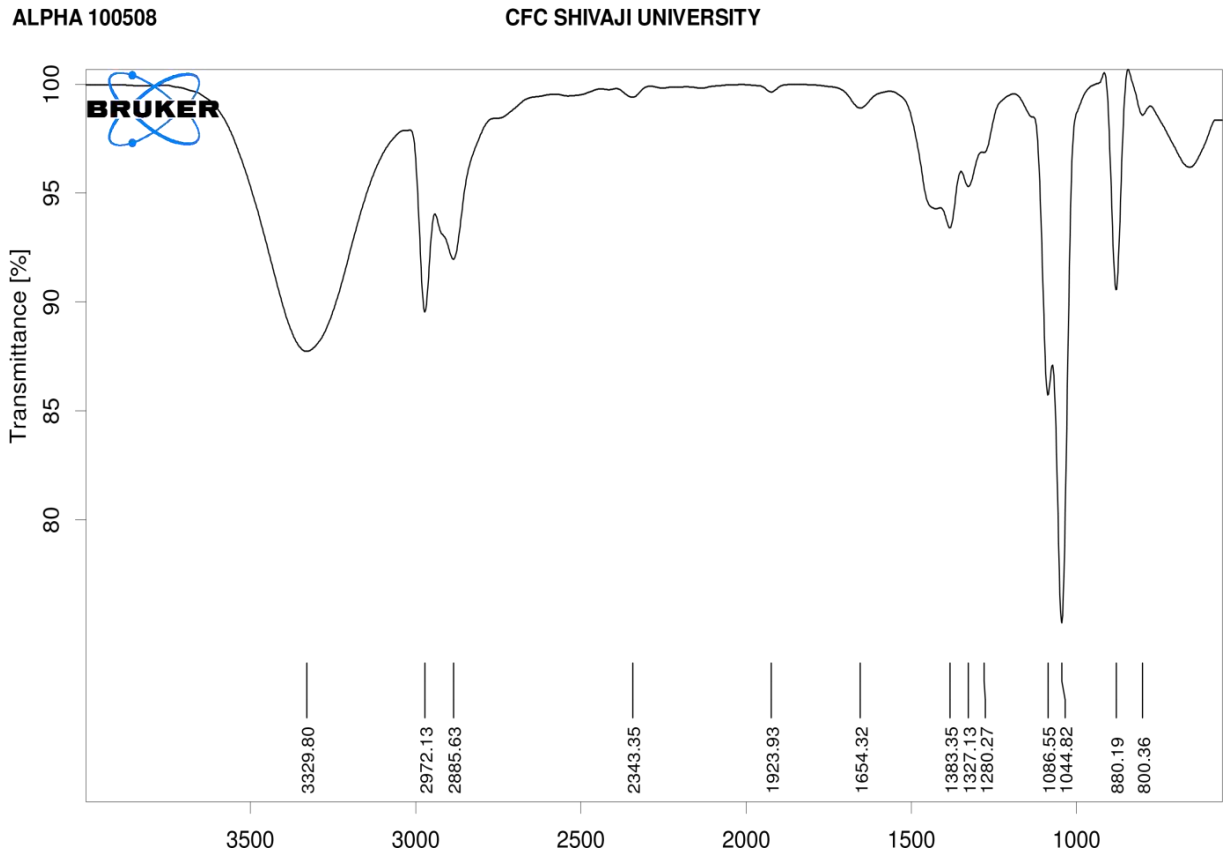


Table10.1

Wavelength($cm^{-1)}$	**Functionalgroup**
880.19	Phenylderivative
1086.55	Alcoholandphenol
2972.13	Carboxylicacid
3329.80	Amine

Fraction 45

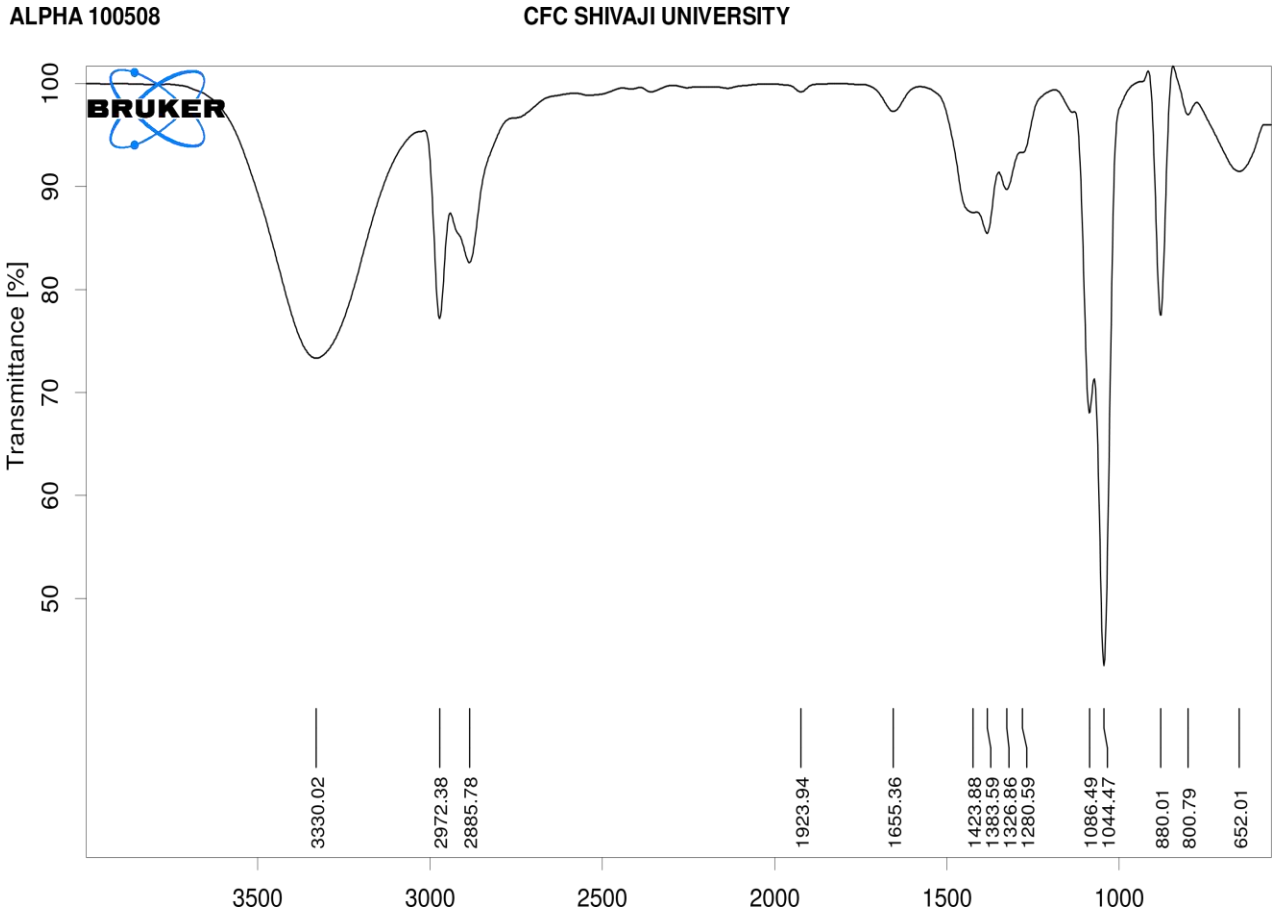


Table 10.2

Wavelength(cm^{-1})	**Functionalgroup**
880.01	Phenylderivative
1044.47	Alcoholandphenol
2972.38	Carboxylicacid
3330.02	Amine

Fraction 54

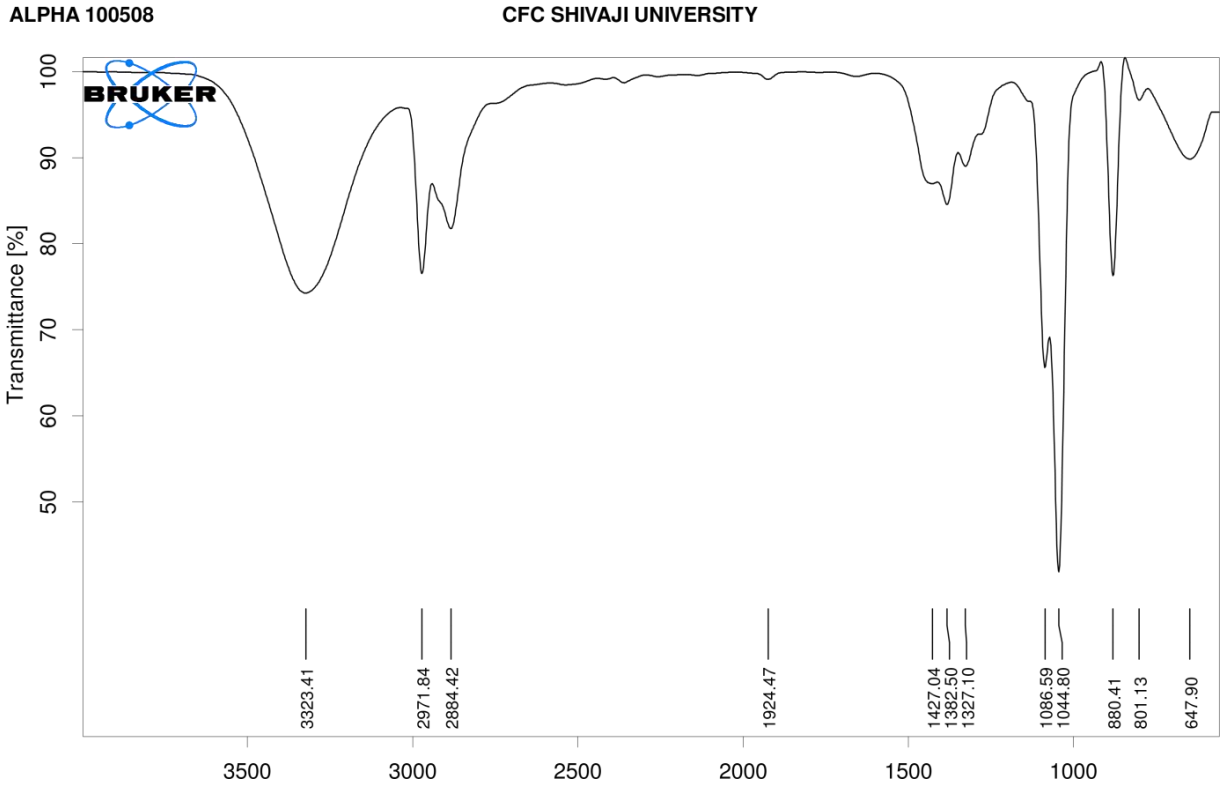


Table 10.3

Wavelength(cm^{-1})	**Functionalgroup**
880.41	Phenyl derivative
1044.80	Alcohol and phenol
2971.84	Carboxylicacid
3323.41	Amine

Fraction 58

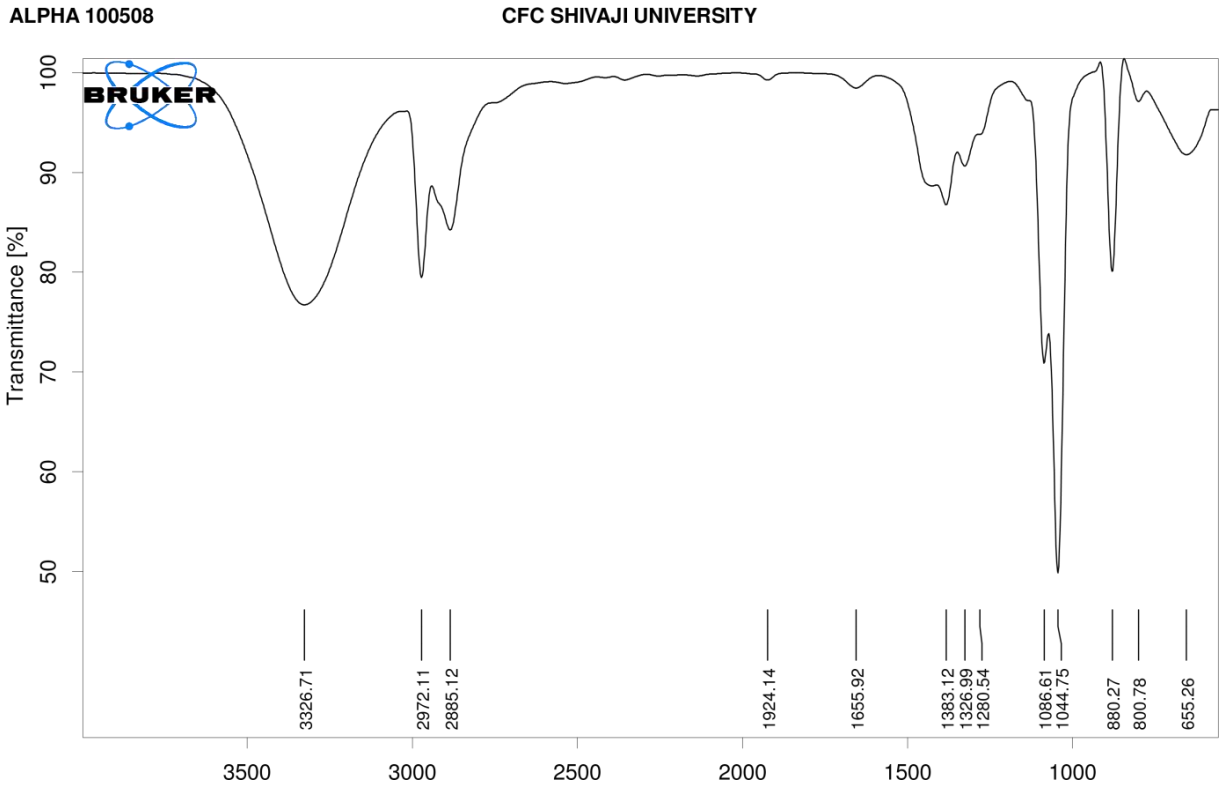


Table 10.4

Wavelength(cm^{-1})	**Functionalgroup**
880.78	Phenylderivative
1044.75	Alcohol and phenol
2972.11	Carboxylicacid
3326.71	Amine

11. Microbialgrowthcurveanalysis:

Themicrobialgrowthcurveanalysisof*pseudomonasaeroginosa* and*staphylococcus aureus* with liquorice plant (stem) extract and without extract.from this experiment we observe that more bacterial growth without extract or less bacterial growth with extract.

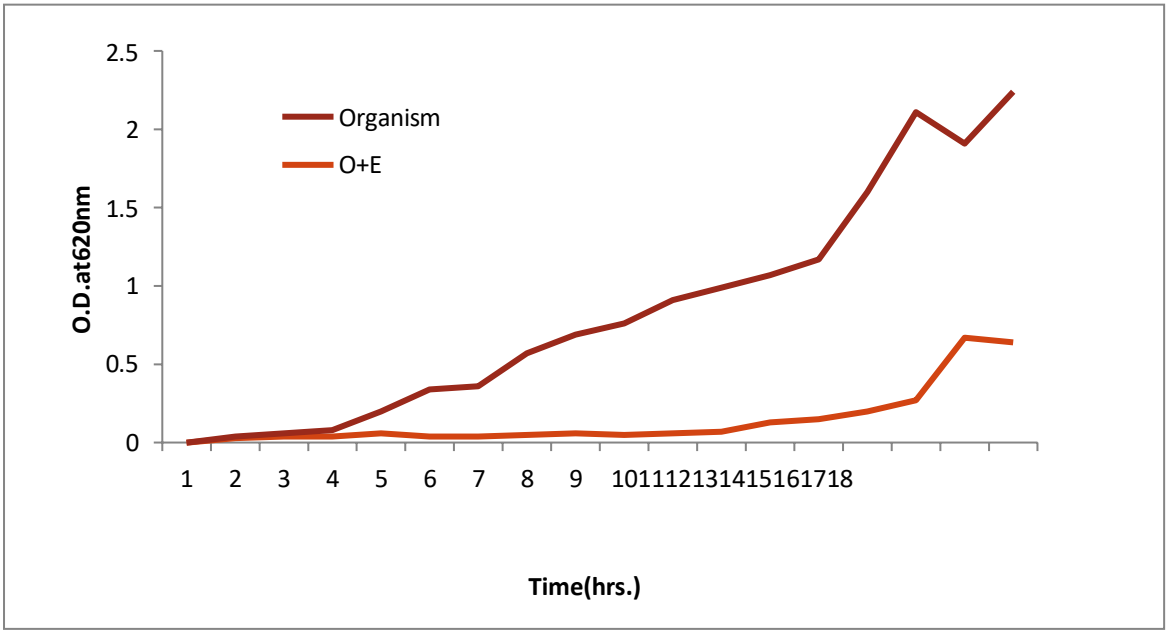


FIG. Growth curve analysis of *pseudomonas aeroginosa* with extract and withoutextract

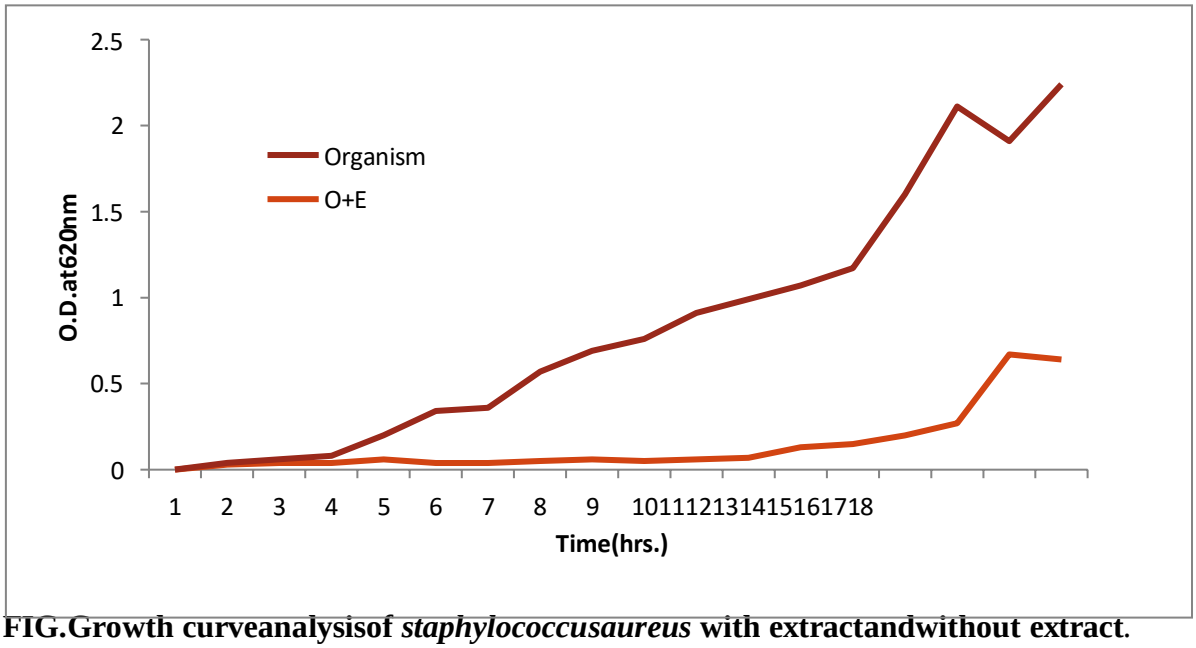


FIG.Growth curveanalysisof *staphylococcusaureus* with extractandwithout extract.

Conclusion:

This plant has been broadly used as a traditional medicine and food industry ingredient, particularly as a flavour and sweetening agent. The stem of plant (*Glycyrrhiza glabra*) tested for phytochemical analysis, antimicrobial activity and identification of phytochemicals by GC-MS.The microbial growth kinetics was studied against plant (stem)extract by using organisms like *pseudomonas aeruginosa* and *staphylococcus aureus*. In present investigation of Phytochemical analysis showed presence of Alkaloids, Diterpenes, Glycosides and Resins.The extract showed Antimicrobial activity against *pseudomonas aeruginosa* and *staphylococcus aureus* organisms. As the current information it is evident that Liquorice has pharmacological function It is also possible that Liquorice might be useful in the development of new drugs for the treatment of various disease.

The compounds separated from this plant (stem) extract like beta.-Tocopherol, O-methyl, alphaamyrin, alpha-tocospiro B and betulinaldehyde which has biological significance like antioxidant activity,anti-inflammatory activity and effective to treat lung cancer. So this plant is medicinally more important.

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