

## BIOLOGICAL EFFECT OF EXTRACTS DRACAEN CINNABARI BALF.F RESIN IN SOCOTRA ISLAND (YEMEN)

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### ABSTRACT

Dragon's blood plant found in various regions around Yemen used to treat various diseases using the resin of the plant. The literature survey reveals that various solvent extracts of the resin have biological activities including anti-microbial and anti-oxidant activities. The various molecules have already been isolated was elucidated from the resin extracts of polar solvents. For the first time we made an attempt to isolate bioactive molecule from the hexane extract. We were successful in isolating and purifying the compound from the crude hexane extract of resin which was showing anti-microbial and anti-inflammatory properties.

**Keywords:** Anti-microbial activity, Dragon cinnabari Blaf.f, Pla2 Enzyme, plant extract.

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## INTRODUCTION

Drugs which are in use presently for many illnesses such as management of pain and inflammatory conditions possess multiple side effects. This provides a deep insight into the use of extracts from plants with their bioactive principles for therapeutic purpose and a totalitarian approach as emphasized in traditional medicine. Natural products that are available in plenty continue to offer major opportunities for finding novel bioactive compounds and provide greater structural diversity than standard combinatorial chemistry. It is thus necessary that the valuable therapeutic properties of newer medicinal plants should be subjected to scientific testing (Cowan MM, 1999), to develop more effective and cheaper drugs. There are two types of plant chemicals, primary metabolites such as sugars, proteins, amino acids, chlorophylls etc. and the other group of chemicals called secondary metabolites, which includes alkaloids, terpenoids, saponins and phenolic compounds. These phytochemicals can exert significant effect as therapeutic agents on the mammalian system. The search for bioactive molecules from one such medicinal plant, plant of panacea, the resin of which are extensively used in curing various types of illnesses was achieved by repeated bioactivity-guided fractionation followed by elucidation of structure using appropriate chromatographic and spectroscopic techniques. In the present investigation research, we focused on isolating anti-

inflammatory and anti-microbial molecules from different solvent extracts of resin of dragon's blood plant. Extracts of resin of *Dracaena cinnabari* with different solvents of increasing polarity exhibited various bioactive properties. Even though hexane extract has shown lesser antimicrobial and anti-inflammatory activity when compared to other solvent extract, it is selected for isolation of molecules because work so far reveals no molecule has been isolated from hexane extract. Thus to explore the rationale behind the use of extracts of *Dracaena cinnabari* for therapeutic purpose in traditional medicine the solvent extracts were subjected for scientific scrutiny.

### History of the dragon's blood plant

Dragon's blood tree is a non-specific name for dark red resinous exudations from different plant species endemic to various regions around globe that belongs to four genera *Dracaena* spp. (Agavaceae), *Croton* spp. (Euphorbiaceae), *Daemonorops* spp. (Palmaceae) and *Pterocarpus* spp. (Fabaceae) have a long history of being used as a traditional medicine the world over (Mallikharjuna, et al., 2007): Medicinal use of dragon's blood dates back to the Ancient Greeks, Romans, Chinese and Arabs (Mothana et al., 2006). However, *Dracaena cinnabari* Balf. f. (*D. cinnabari*) belongs to Agavaceae family, which is commonly known as Damm Alakhwain in Yemen. It is endemic to the Socotra Island, Yemen. *D.*

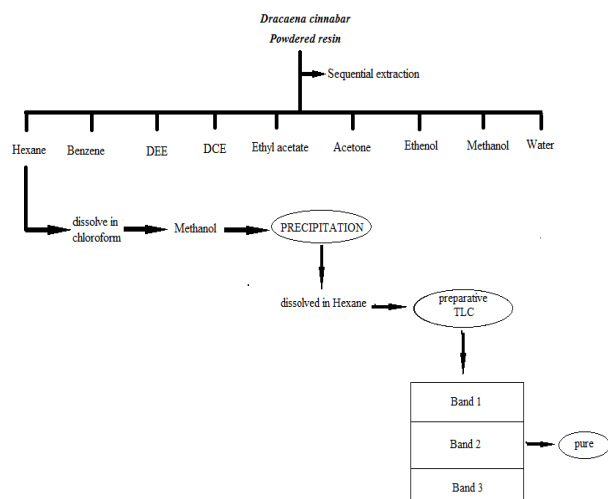
*cinnabari* resin has traditionally been used to treat diarrhea, wounds, fevers, ulcers, haemorrhage, control bleeding, fractures, and burns (Xin et al., 2011). In China, *Daemonorops spp.* and *Dracaena spp.* dragon's blood resin has been used in traditional Chinese medicine to stimulate circulation, control bleeding, treat pain, promote tissue regeneration, wounds, diarrhea, and piles and assist the healing of fractures (Deepika et al., 2009). *Croton spp.* dragon's blood resin is a household remedy in Latin American countries, where it is used to treat diarrhea, bone fractures, hemorrhoids, and cholera. So, in this we are going to isolate anti-inflammatory and anti-microbial molecules from different solvent extracts of resin of dragon's blood plant.



**Figure 1:** Dragon's blood tree (*D. cinnabari* Balf. f.) in Socotra Island, Yemen

### Isolation and purification of a molecule from Dragon's blood plant

Dry powder of the resin of *Dracaena cinnabar* was taken in soxhlet apparatus and subjected for sequential extraction of solvents from non-polar to polar end using hexane, benzene, diethyl ether, dichloromethane, chloroform, ethyl acetate, acetone, ethanol, methanol and water. All the extracts were subjected to PLA<sub>2</sub> inhibition assay and anti-microbial assays respectively. Even though hexane extract has shown lesser activity when compared to other solvent extract, it is selected for isolation of molecules because so far work have been done on rest of the solvent extracts except the hexane extract. Further hexane extract is dissolved in chloroform then different molecules are isolated using the methanol which acts as precipitating agent. The precipitate was separated by filtering with Whatmann filter paper No.1. then the precipitate is dissolved in hexane with little warming. Then preparative TLC is performed on the solution with chloroform as the solvent system. Three bands were seen in which the middle band contains a pure molecule.



**Fig2:** Schematic representation of the procedure for the isolation and purification of molecules

### Preparative thin layer chromatography

Preparative-layer chromatography (PLC) is an effective and easy means of obtaining small quantities of compounds from natural mixtures, which can then be used for different purposes for example determination of the structure of the compounds isolated by spectroscopic methods, or investigation of their biological activity (Hajnos et al., 2006). It should be also remarked that PLC can be used not only for isolation but also for on-line purification of plant extracts rich in non-polar (lipids, chlorophylls, waxes) or polar (tannins, sugars) ballast (Hajnos et al., 1992 and Hajnos et al., 2006), Also can be used as a method of sample preparation, when purification in one step is not sufficient for isolation of a fraction before GC, HPLC, or TLC analysis (Glowniak et al., 2004, Zwickenpflug et al., 1998, Huck et al., 2000, Lin et al., 2000, Wwarzynowicz et al., 1990

and Sharmamet al 1997 ).Preparative-layer chromatography can be also used as a pilot technique for preparative column chromatography, in which both optimization of system selectivity and determination of the effects of overloading are important (Bernart et al., 1997).

The most important aspect of optimization of preparative-layer chromatography is, of course optimization of the chromatographic system. The best chromatographic system depends on the chemical properties of the compounds being separated. The mobile phase should consist of volatile solvents of low viscosity which are easily removed. Buffers, ion-pair reagents, and other components difficult or impossible to evaporate should be eliminated. For basic compounds it is usually necessary to use aqueous ammonia or short-chain amines as additives. The adsorbent should not react irreversibly with the components being separated. The stationary phases used in PLC are similar to those applied in analytical TLC. Normal-phase systems are preferred for preparative purposes. To achieve satisfactory separation in preparative-layer chromatography the effects of a few strategic conditions must be investigated (Glowniak et al., 1987). These include the kind of overloading (volume or mass) and the method of introduction of large volumes of sample to the adsorbent layers.

### Inflammation

Tissue damage caused by a wound or by an invading pathogenic microorganism induces a complex sequence of events collectively known as the inflammatory response (Quby 2002). As described above, a molecular component of a microbe, such as LPS, may trigger an inflammatory response via interaction with cell surface receptors. The end result of inflammation may be the marshalling of a specific immune response to the invasion or clearance of the invader by components of the innate immune system. Many of the classic features of the inflammatory response were described as early as 1600 BC, in Egyptian papyrus writings. In the first century AD, the Roman physician Celsus described the “four cardinal signs of inflammation” as rubor(redness), tumor(swelling), calor(heat), and dolor(pain). In the second century AD, another physician, Galen, added a fifth sign: functiolaesa(loss of function).

### **ANTI-INFLAMMATORY ASSAY**

Tissue damage caused by a wound or by an invading pathogenic microorganism induces a complex sequence of events collectively known as the inflammatory response. As described above, a molecular component of a microbe, such as LPS, may trigger an inflammatory response via interaction with cell surface receptors. The end result of inflammation may be the marshalling of a specific immune response to the invasion or clearance of the invader by components of the innate immune system.

Inflammation, a response triggered by damage to living tissues. The inflammatory response is a defense mechanism that evolved in higher organisms to protect them from infection and injury. Its purpose is to localize and eliminate the injurious agent and to remove damaged tissue components so that the body can begin to heal. The response consists of changes in blood flow, an increase in permeability of blood vessels, and the migration of fluid, proteins, and white blood cells (leukocytes) from the circulation to the site of tissue damage. An inflammatory response that lasts only a few days is called acute inflammation, while a response of longer duration is referred to as chronic inflammation (Quby 2002 and Dery et al., 2004).

### **PLA2 ENZYME**

Phospholipases A<sub>2</sub> (PLA<sub>2</sub>s) are enzymes that release fatty acids from the second carbon group of glycerol. This particular phospholipase specifically recognizes the sn-2 acyl bond of phospholipids and catalytically hydrolyzes the bond releasing arachidonic acid and lysophospholipids. Upon downstream modification by cyclooxygenases, arachidonic acid is modified into active compounds called eicosanoids. Eicosanoids include prostaglandins and leukotrienes, which are categorized as anti-inflammatory and inflammatory mediators (Dery et al., 2004). PLA<sub>2</sub> are commonly found in mammalian tissues as well as insect and snake venom (Mathison et al., 2006) Venom from both snakes and insects is largely composed of melittin, which is a stimulant of PLA<sub>2</sub>. Due



to the increased presence and activity of PLA2 resulting from a snake or insect bite, arachidonic acid is released from the phospholipid membrane disproportionately. As a result, inflammation and pain occur at the site (Mathison et al., 2001). There are also prokaryotic A2 phospholipases.

### Anti-microbial assay

Medicinal plants represent rich source of antimicrobial agents. Plants are used medicinally in different countries as source of many potent and powerful drugs. A wide range of medicinal plant parts is used to extract the raw drugs and possess varied medicinal properties (Mahesh et al., 2008). The different parts used include root, stem, flower, fruit, twinges exudates and modified plant organs. The antimicrobial activity of plant constituents varies with plants part even and these constituents shows different inhibitory response among pathogenic bacteria, yeast and fungi. The potency of plant constituents can be determined by performing microbiological assay. The different plant extracts were found to be active against *Staphylococcus aureus*, *Staphylococcus epidermis*, *Vibrio parahaemolyticus* and *E. coli*. The most important property of an antimicrobial agent, from a host point of view, is its selective inhibition, i.e., the agent acts in some way that inhibits or kills bacterial pathogens but has little or no toxic effect on the host (Ahmad et al., 2007). This implies that the biochemical processes in the bacteria are in some way different from those in the animal cells, and

that the advantage of this difference can be taken in chemotherapy (Yi T et al., 2011). Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial compound that inhibits the visible growth of a microorganism after overnight incubation. MIC values can be determined by a number of standard test procedures. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents (Deepike et al., 2011).

Antimicrobial susceptibility testing methods are divided into two types based on the principle applied in each system (Abu-Taleb et al., 2013). They include disc diffusion (Stokes method and Kirby-Bauer method) method and dilution (Broth dilution and Agar dilution) method. The most commonly employed method is disc diffusion method. Antimicrobial agents are any chemical or biological agents that either destroy or inhibit the growth of microorganisms. Some antibacterial agents are:

#### **E.coli**

*E.coli* is a Gram-negative (bacteria which do not retain crystal violet dye), facultative anaerobic (that makes ATP by aerobic respiration if oxygen is present, but is capable of switching to fermentation or an aerobic respiration if oxygen is absent) and non sporulating bacteria. Cells are typically rod-shaped, and are about 2.0 micrometres ( $\mu\text{m}$ ) long and 0.25–1.0  $\mu\text{m}$  in diameter; with a cell volume of 0.6–0.7  $\mu\text{m}^3$ . It can live on a wide

variety of substrates (Abu-Taleb et al., 2013).

### ***Staphylococcus aureus***

*Staphylococcus aureus* is a gram-positive cocci bacterium that is a member of the Firmicutes, and is frequently found in the human respiratory tract and on the skin (Dharmarha et al., 2008). It is positive for catalase and nitrate reduction. Although *S.aureus* is not always pathogenic, it is a common cause of skin infections (e.g. boils), respiratory disease (e.g. sinusitis), and food poisoning. Disease-associated strains often promote infections by producing potent protein toxins, and expressing cell-surface proteins that bind and inactivate antibodies. The emergence of antibiotic-resistant forms of pathogenic *S. aureus* (e.g. MRSA) is a worldwide problem in clinical medicine

### ***Staphylococcus epidermis***

*Staphylococcus epidermidis* is a Gram-positive bacterium, and one of over 40 species belonging to the genus *Staphylococcus*. It is part of the normal human flora, typically the skin flora, and less commonly the mucosal flora. Although *S. epidermidis* is not usually pathogenic, patients with compromised immune systems are at risk of developing infection (Kluytmans et al., 1997). These infections are generally hospital-acquired. *S. epidermidis* is a particular concern for people with catheters or other surgical implants because it is known to form biofilms that grow on these devices. Being part of the

normal skin flora, *S. epidermidis* is a frequent contaminant of specimens sent to the diagnostic laboratory. This occurs most commonly on intravenous catheters and on medical prostheses (Cole et al., 2001). Infection can also occur in dialysis patients or anyone with an implanted plastic device that may have been contaminated. It also causes endocarditis, most often in patients with defective heart valves. In some other cases, sepsis can occur in hospital patients (Queck et al., 2008 and Hedin et al., 1993).

### ***Vibrio parahaemolyticus***

*Vibrio parahaemolyticus* is a curved, rod-shaped, Gram-negative bacterium found in brackish saltwater, which, when ingested, causes gastrointestinal illness in humans. *V. parahaemolyticus* is oxidase positive, facultative aerobic, and does not form spores (Kita et al., 1973). Like other members of the genus *Vibrio*, this species is motile, with a single, polar flagellum (Falkow et al., 2004).

### **Antifungal activity**

Antifungals are used to kill or prevent further growth of fungi. In medicine, they are used as a treatment for infections such as athlete's foot, ringworm and thrush and work by exploiting differences between mammalian and fungal cells. They kill off the fungal organism without dangerous effects on the host. Unlike bacteria, both fungi and humans are eukaryotes. Thus, fungal and human cells are similar at the

molecular level, making it more difficult to find a target for an antifungal drug to attack that does not also exist in the infected organism. Consequently, there are often side effects to some of these drugs. Some of these side effects can be life-threatening if the drug is not used properly.

### **Candida albicans**

*Candida albicans* is a diploid fungus that grows both as yeast and filamentous cells and a causal agent of opportunistic oral and genital infections in humans, and *Candidal onychomycosis*, an infection of the nail plate. Systemic fungal infections (fungemias) including those by *C.albicans* have emerged as important causes of morbidity and mortality in immunocompromised patients (e.g., AIDS, cancer chemotherapy, organ or bone marrow transplantation). *C. albicans* biofilms may form on the surface of implantable medical devices. In addition, hospital-acquired infections by *C. albicans* have become a cause of major health concerns ("BBB-Bad Bug Book .,2009 and "Dr.Weil's.,2010).

### **MATERIALS**

Different solvent extracts of dragon's blood plant, alpha glucosidase enzyme, PNP-alpha-glucopyranoside, polypropylene cuvette, phosphate buffer saline (50mM pH 6.8), nutrient agar, 1XTAE buffer, EDTA, agarose, ethidium bromide, PLA2 enzyme, human erythrocytes, egg yolk, TLC plates, silica gel G mesh size (100-200), chloroform.

### **METHODOLOGY**

#### **Preparation of resin powder and extraction**

The powdered resin 500 g was extracted with different solvents depending on the polarity of the solvents. Started with non-polar to polar (hexane, benzene, diethyl ether, dichloromethane, chloroform, ethyl acetate, acetone, ethanol, methanol and water) by Soxhlet apparatus for 24h for each solvent. Extracts were filtered and concentrated under vacuum in a rotary evaporator. The extracted samples were stored at 4 °C for further use.

#### **Isolation of molecules from hexane extract (*Dracaena cinnabar*)**

500 gm of dry powder of resin of *Dracaena cinnabar* was taken in soxhlet apparatus and subjected for sequential extraction of solvents from non-polar to polar end using hexane, benzene, diethyl ether, dichloromethane, chloroform, ethyl acetate, acetone, ethanol, methanol and water. All the extracts were subjected to PLA<sub>2</sub> inhibition assay and anti-microbial assays respectively. Further hexane extract is dissolved in chloroform, to that methanol was added and the precipitation was got. The precipitate was separated by filtering with Whatman filter paper No.1.then the precipitate was dissolved in hexane on little warming. A preparative TLC was performed to isolate the molecules. The compounds were separated using the solvent system chloroform.

#### **ANTI INFLAMMATORY ACTIVITY**

The PLA<sub>2</sub>, obtained from Russel viper venom were assayed by indirect



haemolytic activity using the method of Boman and Kaletta (Boman HG and Kaletta, 1957). Briefly, packed human erythrocyte, egg yolk and phosphate-buffered saline (1: 1: 8, v/v) were mixed. One ml of thus obtained suspension was incubated with the enzyme (60µg), which was pre incubated with compounds of different concentrations for 10 min at 37 °C. The reaction was stopped by adding 9 ml of ice-cold phosphate-buffered saline, the reaction mixture was centrifuged at 4 °C for 10 min at 2000 rpm. The amount of haemoglobin released in the supernatant due to haemolysis was measured at 540 nm. The enzyme- substrate mixture was used as positive control. Values are presented as the mean of 4 independent determinations.

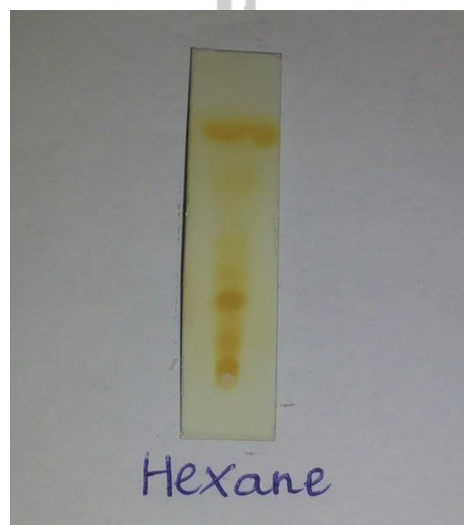
#### ANTI-MICROBIAL ASSAY

The different solvent extracts of dragon's blood plant were evaluated *in vitro* for antibacterial activity against *Staphylococcus aureus*(SA), and *Staphylococcus epidermis*(SP) as examples of Gram-positive bacteria and *Vibrio parahaemolyticus* and *Escherichia coli* (EC) as examples of Gram-negative bacteria and *Candida albicans* as fungi. Inhibition zone diameter (IZD) in mm was used as criterion for the antimicrobial activity using agar diffusion well method. Ampicillin were used as reference drugs for antibacterial activity. Microbes were grown in nutrient broth (NB, Merck) medium at 37°C for 22h. The bacterial number in the final inoculums was adjusted to 10<sup>6</sup> CFU/ml. A bacterial lawn was prepared by pouring 0.1 ml of bacterial

suspension onto each plate of nutrient agar medium (NA, Merck), spread by a sterile cotton swab, and allowed to remain in contact for 1 min. Different solvent extracts of different concentrations were prepared in order to impregnate the paper discs. The sterile filter paper discs containing Schiff base derivatives (6-mm diameter) were then placed on the bacterial lawn. The petri dishes were subsequently incubated at 37°C for 24 h and the inhibition zone around each disc was measured in mm. Gentamicin and fluconazol were used as positive controls.

## RESULTS AND DISCUSSION

### Thin layer chromatography

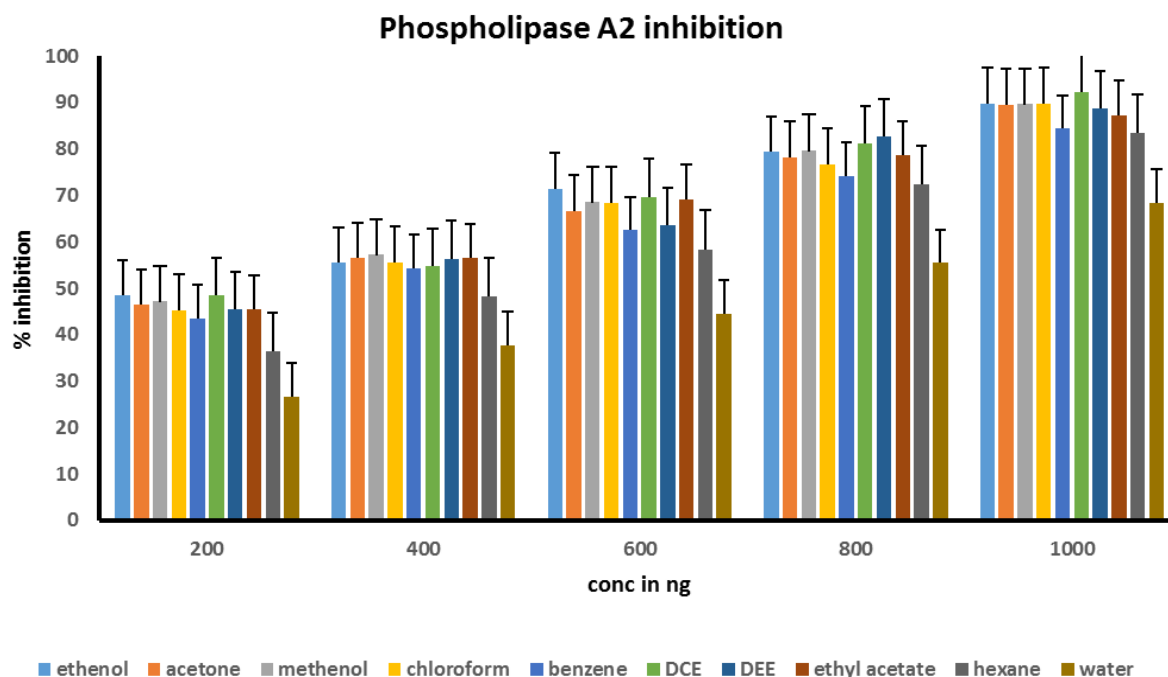


**Fig.3a:** Micro TLC of hexane extract of resin showing 6 different spots on separation using the solvent system ethyl acetate: chloroform: methanol (1:1:1 v/v).

The crude hexane extract of the resin when treated with methanol precipitation was obtained. The precipitate was loaded on to silica gel column with a mesh size of 100-120. The column was packed in chloroform and the sample was eluted using chloroform. Resulting eluent was separated on TLC using the solvent system chloroform. Three bands were obtained, the middle yellow coloured band was scraped and dissolved in chloroform and centrifuged at 2000rpm for 10mins and the supernatant was reloaded on to the micro TLC plate and developed using the solvent system chloroform, single spot was obtained as shown below.



**Fig. 3b:** TLC plate with single spot after the separation using chloroform as a solvent system.



**Figure5b:** The inhibitory effects of different solvent extracts of Dragon blood resin (Ethanol, Acetone, Methanol, Chloroform, Benzene, DCM, DEE, Ethyl acetate, Hexane, Water) on PLA2 activity.

Different solvent extracts of resin	Ethanol	Acetone	Methanol	Chloroform	Benzene	DCM	DEE	Ethyl acetate	Hexane	Aqueous
IC50 value (ng/ml)	250ng	295ng	270ng	320ng	330ng	260ng	295ng	295ng	470ng	750ng

**Table 1:** inhibition of the PLA2 by different solvent extracts of the resin with their IC50 value.

### ANTI-INFLAMMATORY ACTIVITY

PLA2 inhibition by different solvent extracts of Dragon blood resin. PLA2 is commonly found in mammalian tissues as well as in insect and snake venom. Venom from both snakes and insects is largely composed of melittin, which is a simulant of PLA2. Due to the increased presence and activity of PLA2 resulting from a snake or insect bite, arachidonic acid is released from the phospholipid membrane disproportionately. As a result, inflammation and pain occur at the site. In vitro studies of the different solvent extracts of the resin of Dragon's blood plant demonstrated that the solvent extracts

inhibited the PLA2 enzyme that is responsible for inflammation. The inhibition increased gradually with increase in the concentration of the extracts and had no adverse effects as that of the Non-steroidal Anti-inflammatory drugs (NSAID's) as the extracts are plant based.

Ethanol extract has showed the maximum inhibition with a IC50 value of 250ng/ml which is followed by DCM and Methanol extract with 260 and 270ng/ml, acetone chloroform, benzene, DEE, ethyl acetate and hexane extracts have shown moderate inhibition with IC50 value of 295, 320, 330, 295, 295 and 470ng/ml respectively, aqueous extract did not show significant inhibition. /ss[poiu321q

### ANTI-MICROBIAL ACTIVITY

Organisms	E.coli	S.aureus	S.epidermis	v.parahaemolyticus	Candida albicans
<b>MIC Values in µg/mL</b>					
Gentamicin	7.26	9	6.8	10	8.6
Fluconazole	----	----	----	----	0.75
Ethanol extract	31.25µg	35.71µg	22.72µg	35.71µg	22.72µg
Acetone	27.77µg	25µg	35.71µg	41.66µg	50µg

extract					
<b>Methanol extract</b>	31.25µg	35.71µg	22.72µg	27.77µg	41.66µ
<b>Chloroform extract</b>	19.23µg	25µg	27.77µg	22.72µg	25µg
<b>Benzene extract</b>	16.66µg	19.23µg	19.23µg	27.77µg	27.77µg
<b>DCM extract</b>	20.83µg	50µg	16.66µg	19.23µg	27.77µg
<b>DEE extract</b>	20.83µg	20.83µg	16.66µg	20.83µg	13.88µg
<b>Ethyl acetate extract</b>	50µg	25µg	35.71µg	41.66µg	50µg
<b>Hexane extract</b>	50µg	--	--	41.66µg	50µg
<b>Aqueous extract</b>	--	--	--	--	107.14µg

**Table 2:** Anti microbial activity of different solvent extracts of Dragon's blood resin.

Extracts (Ethanol, Acetone, Methanol, Chloroform, Benzene, dichlorobenzene (DCM), diethyl ether (DEE), Ethyl acetate, Hexane, aqueous) were tested in vitro for their anti-microbial activity against, two Gram-negative bacterial strains and a fungal strain. Commercial antibiotics such as gentamycin and fluconazole were used as standard drugs. The results were compared with standard drugs and depict in table 1.

DEE extract was found to be more potent against Gram-positive (*S.aureaus*, *S.epidermis*) Gram-negative bacteria

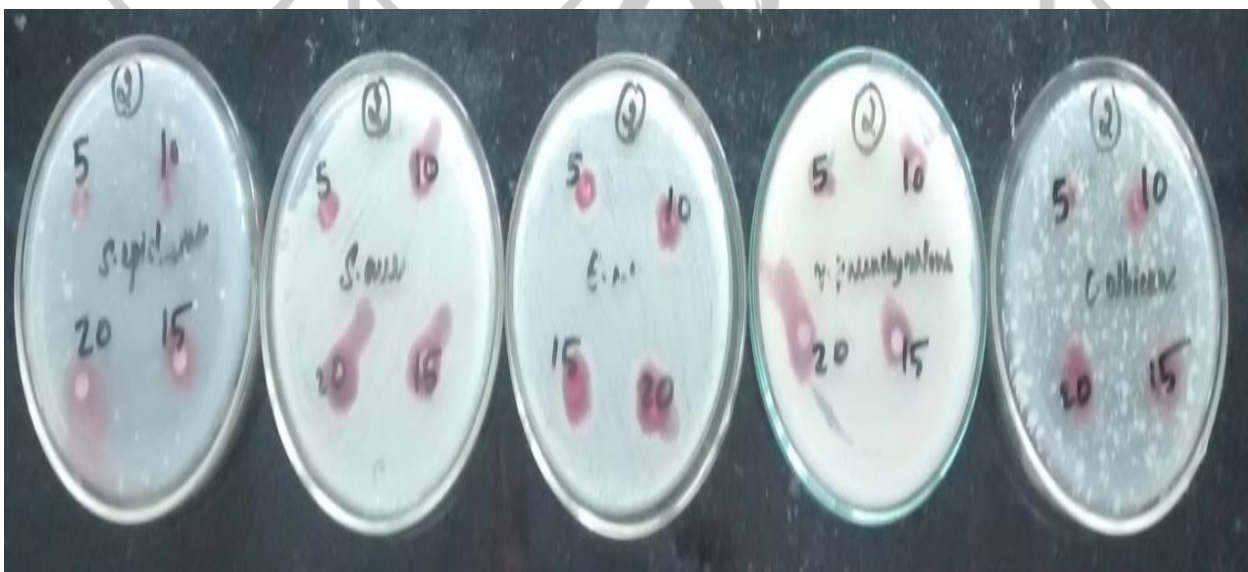
(*E.coli* and *V.parahaemolyticus*) also for fungi (*Candida albicans*) with the MIC value of 20.µg/mL, 20.83µg/mL, 16.66 µg/mL, 20.83 µg/mL, and 13.88 µg/mL. Benzene extract was more potent on *E.coli* with MIC value of 16.66 µg/mL. Methanol, acetone, ethanol and chloroform exhibited moderate anti-microbial activity. Hexane extract showed moderate activity on gram negative bacteria and anti-fungal activity but did not show any activity on gram positive strains. Of all the compounds aqueous extract did not show any anti-bacterial activity.

**Ethanol extract**



**Fig 3a:** Antimicrobial activity of ethenol extract of *Dracaena cinnabari* on *S.epidermis*, *S.aureus*, *E.coli*, *V.paraahaemolyticus* and *C.albicans*

**Acetone extract**



**Fig 3b:** Antimicrobial activity of acetone extract of *Dracaena cinnabari* on *S.epidermis*, *S.aureus*, *E.coli*, *V.paraahaemolyticus* and *C.albicans*



### Methanol extract



**Fig 3c:** Antimicrobial activity of methanol extract of *Dracaena cinnabari* on *S.epidermis*, *S.aureus*, *E.coli*, *V.parahaemolyticus* and *C.albicans*

### Chloroform extract



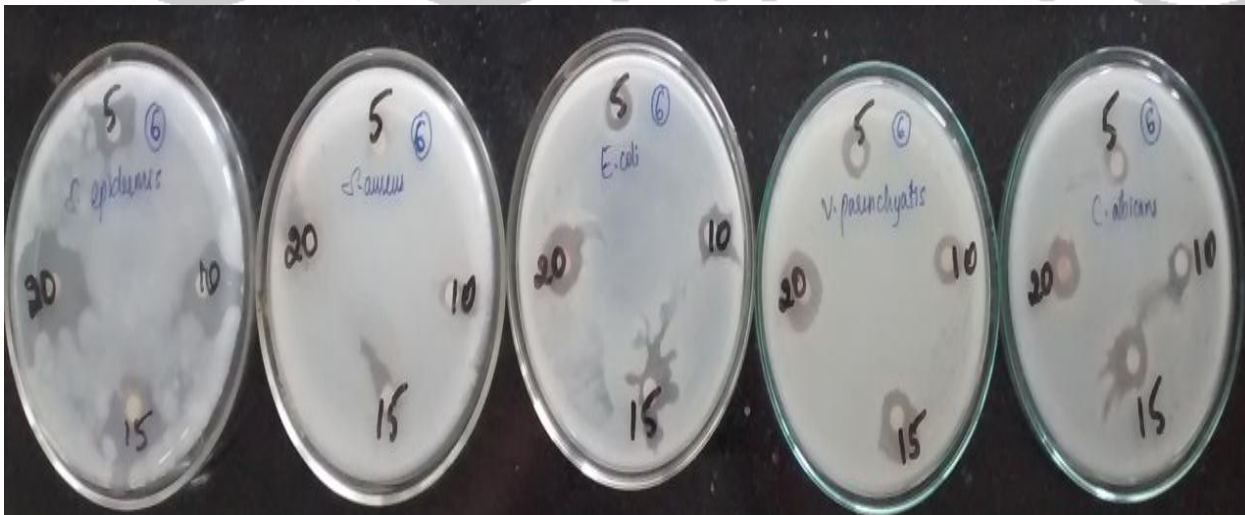
**Fig 3d:** Antimicrobial activity of chloroform extract of *Dracaena cinnabari* on *S.epidermis*, *S.aureus*, *E.coli*, *V.parahaemolyticus* and *C.albicans*

### Benzene extract



**Fig 3e:** antimicrobial activity of benzene extract of *Dracaena cinnabari* on *S. epidermis*, *S. aureus*, *E. coli*, *V. parahaemolyticus* and *C. albicans*

### Dichloromethane (DCM) extract



**Fig 3f:** antimicrobial activity of dichloro ethane extract of *Dracaena cinnabari* on *S. epidermis*, *S. aureus*, *E. coli*, *V. parahaemolyticus* and *C. albicans*

**Diethyl ether extract:**



**Fig 3g:** antimicrobial activity of di ethyl ether extract of *Dracaena cinnabari* on *S.epidermis*, *S.aureus*, *E.coli*, *V.parahaemolyticus* and *C.albicans*

**Ethyl acetate extract**



**Fig 3h:** antimicrobial activity of Ethyl acetate extract of *Dracaena cinnabari* on *S.epidermis*, *S.aureus*, *E.coli*, *V.parahaemolyticus* and *C.albicans*



**Hexane extract**



**Fig 3i:** antimicrobial activity of Hexane extract of *Dracaena cinnabari* on *S.epidermis*, *S.aureus*, *E.coli*, *V.parahaemolyticus* and *C.albicans*

**Aqueous extract**



**Fig 3j:** antimicrobial activity of aqueous extract of *Dracaena cinnabari* on *S.epidermis*, *S.aureus*, *E.coli*, *V.parahaemolyticus* and *C.albicans*

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## CONCLUSION

In various regions around Yemen, Dragon's blood plant is found. This plant is used to treat various diseases by using the resin of the plant. Various solvent extracts of the resin which have biological activities including anti-microbial and anti-oxidant activities was revealed by the literature survey. The various molecules have already been isolated was elucidated from the resin extracts of polar solvents. For the first time we made an attempt to isolate bioactive molecule from the hexane extract. We were successful in isolating and

purifying the compound from the crude hexane extract of resin which was showing anti-microbial and anti-inflammatory properties, further the molecule need to be subjected for various spectroscopic analysis like IR, NMR, MS and elemental analysis, so the structure could be elucidated.

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