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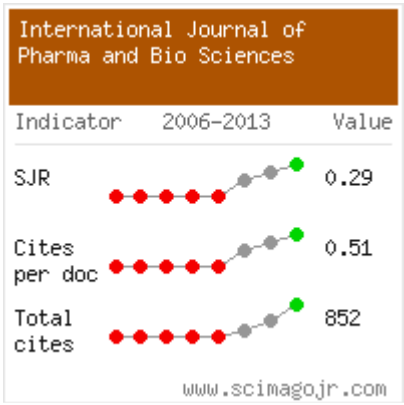

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CONTENT SPECIAL ISSUE

NERVE STIMULATION BASED PROSTHETIC ARM FOR AMPUTEES

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ABSTRACT:

The hand is an important organ of human being. About half the bones in the human body are found in hand and feet. Fingers have an indigenous range of fine, delicate movements. The electric powered hand was developed 50 years ago to grasp. But the advanced hand with microprocessor controls and miniaturized components within the individual fingers helps to deliver finest movements. A bionic arm combines robotics, biotechnology and electronics to recreate the functions of human arm. The advanced bionic arms like bebionic, I limb, Michelangelo arms facilitate the usage of all normal functions of tissue arms. These prosthetic hands are controlled and operated by capturing the Electro Myo Gram (EMG) signals and Li- ion batteries. These arms have special compartments to store the series of batteries. These hands give the boon to the amputees. This paper explains how to capture the EMG signals from the arms to convert into mechanical action of phalange. We have done a survey over the Advanced BIONIC ARM and noticed a drastic change from the ancient day to the present day. We proposed to develop a prototype of an enhanced bionic arm by capturing the nerve stimulators to activate the prosthetic limbs.

KEYWORDS:

Prosthetic hand, bebionic, Michelangelo, EMG signals, amputees, phalange.

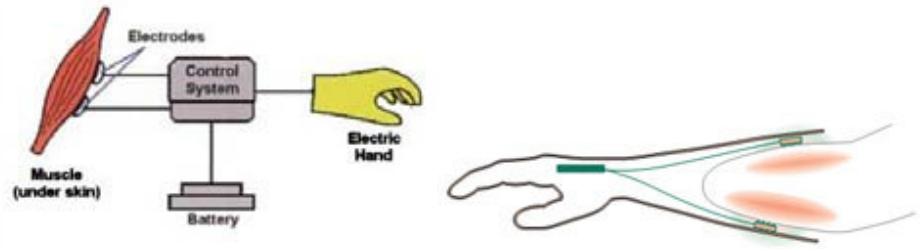
INTRODUCTION:

The world moves on by century to century meanwhile the technology also growing rapidly. Human hand prosthesis is developed in earlier centuries with hooks. It has been upgraded step by step. The first prosthetic limb was developed in earlier stages called civil war prosthetic hook. Later it is developed into the Vincent, i-limb, i-limb pulse, bebionic, bebionic v2 ,and Michelangelo hands. These all prosthetic hands are working with the help of picking up the Myo signals from the amputees muscle. The main components of prosthetic limbs are electrodes, graphical recorder, servo motor, microprocessor and batteries. The function of this hand is to pick up

the electrical signal from the muscle and send it to the microprocessor to stimulate the process as shown in the figure (Fig 1)[1]. With the help of prosthetic hand we can able to hold the common things in daily life such as ball, bat, pen, cube etc.,

Figure 1

Picking up the Myo signals from the muscle and the prosthetic hand socket with electrodes. Courtesy: Muzumdar, 2004.



This paper explains the functions and specifications of the current bionic arms which is commercially available in the market and a new system of prosthesis involving nerve stimulation. We noticed that the existing prosthetic hands are having some disadvantages even though they are working like a original hand because of the less finger grip and thumb rotation. The weight of the bionic hand is also the one disgrace for it due to the batteries and the control components in it. The battery, controller and two force sensing resistors are used to stimulate the electromyography electrodes which all present in the i limb pulse, bebionic v2 hands. So, the nerve stimulation of the bionic hand is much useful to reduce the weight of the anthropomorphic prosthetic limbs by eliminating it (i.e. Myo signal electrode stimulators). We proposed to develop a prototype over the prosthetic limb which is controlled and stimulated by nerve stimulators. This bionic hand helps the amputees to lift the hand and do the work properly as carried out by a normal hand.

MATERIALS AND METHODS:

The components used in the prosthetic hands are sensing electrodes, batteries, microprocessor, motors, electronic speed controller, microcontroller selection, fingertip force sensor, and so on. Let us see the some important components in this hand briefly. The electrodes are specially designed to pick up the Myo signals

effectively and reduced in size to place within the socket [8,9]. The electronic speed controller is used to take power from the battery which is converted into a controllable desired voltage to control a motor's direction and power level. When selecting the proper speed controller, we must look at the required voltage, maximum current drawn, average current drawn and features. In this case, the features required are full forward and reverse (some small speed controllers are meant for single direction only which are designed for remote control airplanes), good low speed control, and PWM input, the remote control and hobby signal input standard. [2]

The Microcontroller is capable of handling all the movements in addition to the sensor inputs and user feedback and the features are: Dimensions: 0.7x1.3" (18x33mm), Atmega328 running at 16MHz with external resonator (0.5% tolerance), USB connection off board, Supports auto-reset, 5V regulator, Max 150mA output, Over current protected, Weighs less than 2 grams!, Reverse polarity protected, DC input 5V up to 12V, On board Power and Status LEDs, Analog Pins: 8, Digital I/Os: 14 (Sparkfun Electronics, 2012) .[2] The fingertip force sensor is used to sense the object and able to apply the grip force with respect to the lifting object.

A motor is the main component of the bionic limb which is responsible for the movement and flexible action of the hand. Achieving a more complex set of movements relies on integration with a digital control method. These can be very basic, such as placing a controlling unit into the user's shoe, or very complex such as myoelectric control that interprets electrical activity in the neuromusculature of the limb stump to allow motion.[3] The reinnervated muscles act as biological amplifiers of motor commands in the amputated nerves and the surface electromyogram (EMG) can be used to enhance control of a robotic arm.[12] The sensors are also play a vital role in sensing the objects to protect the bionic hand from the harm. It is the advanced technology in the current prosthetic limbs.

EXISTING PROSTHETIC LIMBS:

1. VINCENT LIMB:

This is the world's first touch sensing hand prosthesis with least weight and compact design. After the VINCENT systems the first bionic hand was introduced with the six motor control, individual movable

digits, and fully movable thumb for the first time in 2009. This is able to sense the environment like human skin. The sentient prosthesis should stimulate the sensory area of the cerebral cortex by selective stimulation of receptors on the arm stump and thus has a positive effect on phantom pain and also makes gripping of goods easier and safer.[4] Now, VINCENT evolution 2 has been introduced with a compact and biomechanically optimized hand from a high-strength aluminium alloy. It combines 10 bi-directionally motor driven axes with an innovative control strategy which is unique in the field of hand prostheses. The hand allows an active individual agility of the fingers and the thumb. The springs between the proximal and distal joints also allow an adaptive tension - in accordance with muscles and ligaments of the human hand[4]. This hand is working by picking up the muscle potential with the help of electrodes which is located inside the socket.

Figure 2

VINCENT evolution 2 prosthetic limb with label.

This limb helps the amputees to do work with the necessary grip force, sense of touch, and force feedback. A force feedback system has been developed for inclusion as standard in the VINCENTevolution2 prosthetic system which was suitable for everyday use and considerably mitigated this habituation effect.[4]

2. BEBIONIC LIMB: [6]

A bebionic hand uses five actuators and therefore requires a higher current supply than traditional myo-electric hands. This can be best accommodated using bebionic batteries and cabling. These include split cell batteries in 2200mAh or 1300mAh. Each battery is provided with an integrated ON / OFF switch and the required cabling. It has 14 different grip patterns and hand positions to do anything you want in the day to day life like eating meals, carrying bag, typing keyboard, etc., New Seal-in Electrodes are the perfect compliment

for Suction Sockets. They are retrofittable in most sockets and provide increased sensitivity to capture weak signals. This hand can also be configured and customized wirelessly to the user requirements via easy-to-use software package, be balance. Biocompatible titanium skin contacts provide superior conductivity, while interference protection shields from common power source and high frequency emitting devices. This hand is suitable for child and adults.

Enhanced skin contact interface are -

- Superior sensitivity to capture weak signals
- Proportional control and built-in gain adjustment
- Advanced electronics with interference protection
- Available in 50Hz or 60Hz
- Retrofittable in existing sockets.

Figure 3

[5] **(a)** Vincent hand by Vincent Systems, **(b)** iLimb hand by Touch Bionics, **(c)** iLimb Pulse by Touch Bionics, **(d)** Bebionic hand by RSL Steeper, **(e)** Bebionic hand v2 by RSL Steeper, and **(f)** Michelangelo hand by Otto Bock. All hands shown without cosmetic glove.[5]

The individual motors placed in each finger of this limb allow moving and gripping the things. Motors are positioned to optimize weight distribution. Powerful microprocessor continuously monitors the position of the each finger which is responsible for limb movement. Propotional speed control gives precision control over delicate tasks. It has four wrist options such as quick disconnect, Multi-Flex, Flexion and short wrist. Bebalance software and wireless technology located in this bionic limb helps to work efficiently. Selectable thumb positions and built in sensor enable to complete more tasks. Auto grip is used to sense the objects to protect it from damage due to slipping from the hand. Foldaway fingers provide this hand looking like a original human upper limb. Durable construction and advanced material makes this hand strong enough to handle the things up to 45 kg. Innovative palm design and soft finger pads protects bebionic hand from impact damage and makes the hand quieter than ever.

BEBIONIC V2 limbs are also have the same functions and specifications with more efficient than bebionic limb.

3. I LIMB HAND:

The I limb is also a type of prosthetic limb commercially available in the market, which is controlled through the use of myo signals. The electrodes are present inside socket pick up the Myo signals according to the action or movement of the amputees muscle. These signals are sent to the microprocessor which causes the device to move. The I limb hand has up to four different muscle

triggers. They are hold open, double impulse, triple impulse and co-contraction. The features of the I limb hand are-

- Smarter - i-mo technology - use of simple gestures to change grips
- Faster - boost digit speed by up to 30%
- Stronger - up to 30% more power when needed
- Smaller - anatomical styling now available in 3 sizes - smaller size hand suitable for women and children.[7]

The capacity with 1,300 mAh and 2,000 mAh are the two batteries applicable for I limb hands. These batteries are rechargeable with the help of charger. There are four wrist connection options are present in this hand such as Quick wrist connection (QWC), Wrist Disarticulation, Flex Wrist and Multi-flex Wrist. These all connections are controlled by switch which has ON/OFF mode. After the installation of I limb to the amputees it can be covered with the active skin which is looks like a original human skin. The i-limb ultra revolution is fitted with a Bluetooth® receiver enabling it to work with a sophisticated software package known as biosim. biosim-pro is the clinician's version of biosim and biosim-i is the version designed for patient users. Using biosim it is possible to make changes to the functionality of the hand. biosim-i is the simplified patient user version of biosim and contains with access to training and games features along with some basic changes to settings.[7] These biosim method is one of the most advanced technology to control the process of the I limb hand. This hand have the more grip force then the all other bionic hands. Even though it has some defect due to battery problems. I limb pulse are also have the same functions and specifications of the I limb hand with some upgraded technologies i.e. result of this hand is more efficient than the I limb.

4. MICHELANGELO HAND:[2]

The Michelangelo Hand built by Advanced Arm Dynamics is simply the most advanced hand on the market today in prosthetics. It actually has the powered opposable thumb, the first one released as an actual product. Sadly, the arm costs $100,000, so it is unable to be purchased, and difficult for even insurance companies to pay for. (Pittman, 2012) The hand is incredibly well refined and streamlined in execution.

GENERAL CHARACTERISTICS OF COMMERCIAL PROSTHETIC HANDS:

VINCENT HAND: (2010) [8]

1. Developer – Vincent systems
2. No.of joints – 2
3. Degree of freedom – 6
4. No.of actuators – 6
5. Actuation method – DC motor-worm gear
6. Joint coupling method – Linkage spanning MCP to PIP
7. Adaptive grip – yes.

I LIMB: (2009) [9]

1. Developer – Touch bionics
2. No.of joints – 11
3. Degree of freedom – 6
4. No.of actuators –5
5. Actuation method – DC motor-worm gear
6. Joint coupling method –Tendon linking MCP to PIP
7. Adaptive grip – yes
8. Weight (g) – 450-615

I LIMB PULSE: (2010) [9]

1. Developer – Touch bionics
2. No.of joints – 11
3. Degree of freedom – 6
4. No.of actuators –5
5. Actuation method – DC motor-worm gear
6. Joint coupling method –Tendon linking MCP to PIP

7. Adaptive grip – yes
8. Weight (g) – 460-465

BEBIONIC LIMB: (2011) [10]

1. Developer – RSL steeper
2. No.of joints – 11
3. Degree of freedom – 6
4. No.of actuators –5
5. Actuation method – DC motor- lead screw
6. Joint coupling method – Linkage spanning MCP to PIP
7. Adaptive grip – yes
8. Weight (g) – 495 – 539.

BEBIONIC V2 LIMB: (2011) [10]

1. Developer – RSL steeper
2. No.of joints – 11
3. Degree of freedom – 6
4. No.of actuators –5
5. Actuation method – DC motor- lead screw
6. Joint coupling method – Linkage spanning MCP to PIP
7. Adaptive grip – yes
8. Weight (g) – 495 – 539.

MICHELANGELO HAND: (2012) [11]

1. Developer – Otto block
2. No.of joints – 6
3. Degree of freedom – 2
4. No.of actuators –2

5. Actuation method – -
6. Joint coupling method – cam design with liks to all fingers
7. Adaptive grip – no
8. Weight (g) – ~420.

CONCLUSION:

The current prosthetic hands are all having the similar function with some changes such as weight, grip force, actuation method and batteries. These all bionic limbs are controlled and processed by only conducting the Myo signals from the muscle of amputees. So, the bionic limbs have electrodes and batteries to stimulate processor with proper cabling. This takes the 50% weight of the limb. We propose the nerve stimulation based prosthetic arm which helps to reduce the weight of the limb because it doesn't need this much battery capacity and components. The nerves are able to polarize and depolarize, according to the brain stimulation. So, we directly connect the processor with nerve stimulators in addition with the amplifier. This technique will give better result than the existing prosthetic limbs. We focused to develop a prototype over the nerve stimulation prosthetic limb in upcoming years.

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IMAGE SEGMENTATION AND MATCHING BASED DENTAL BIOMETRIC SYSTEM- A SURVEY

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ABSTRACT:

Biometric is an identification tool with wider applications. This Biometric identification system is based on physical characteristics. In the past few years dental biometric has emerged as vital biometric information for the human beings on the basis of its stability, uniqueness and contours of teeth. It uses dental photograph and dental radiograph technique for human identification. These systems are used during the Ante mortem (AM) and Post mortem (PM) to identify unidentified subject. The Dental biometric involves three processes preprocessing of dental radiography, segmentation and matching of AM and PM radiography. Feature extraction method uses anisotropic diffusion method to enhance the dental image with a mixture of Gaussian model to separate the dental image. Matching process is used to get the acquired data from the process and match the similarities between two images in identification of human beings. Matching of AM with PM can be done by using specific algorithm. This paper surveys different techniques used in dental biometric.

KEYWORDS:

Biometric, Dental biometrics, segmentation, Dental radiograph, Dental photograph, Matching.

1. INTRODUCTION:

Bio metric relates to human physiological characteristics (or) it is the application of statistical analysis of biological data. It is used to identify individuals from groups. In human different aspects like human physiology and behavior can be used for biometric authentication. According to physiological character, they are related with the shape of the body like finger print, palm vein, face recognition, iris recognition, dental recognition etc. Behavioral characteristics are related with the voice, gait and pattern of behavior of the person.

Since every human in this world has different biometric characteristics we can use this unique feature to identify a person. Our paper is to identify the particular person using the dental biometric system when

compared with its database images. So this process is mainly used in forensic sciences.[4] The main purpose for using this technique in forensic dentistry is to identify the deceased individual.

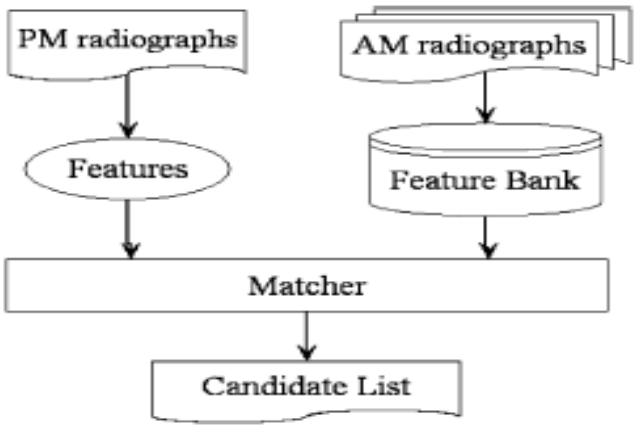


Figure 1: Basic block diagram of Identification technique using Ante mortem records and post mortem records

This can be done by comparing the Post mortem (PM) dental records with the Ante mortem (AM) records to identify the closest match. These are the two main advantages of using this technique. Firstly, it will compare the PM record with the AM record with multiple identities to get the closest match. Secondly, manual system is used when there are a less set of data to analyze and verify whereas automatic system is used to identify on a large database.[1]

1.1 DENTAL BIOMETRIC:

Teeth has a unique identification system due to containing various contours and their mode of arrangement. Teeth are a part of human organ which are made of calcium and that are not easily decayed even after the death of human beings. According to this paper, the technique proposed uses features like tooth present/not present, crown and root morphology, dental restoration, tooth contours etc.[1, 2] This technique for identification uses three main steps: Preprocessing, Segmentation and Matching.

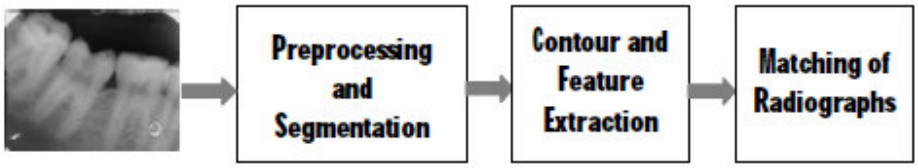


Figure 2: basic block diagram of dental biometric system

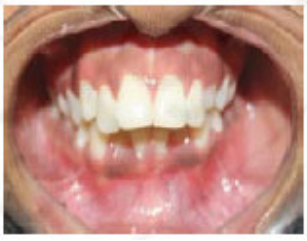
Preprocessing is a technique used to get output from the input ant this output is used as an input in other process.[9] Segmentation usually uses threshold operation to separate the desired dental work.[9] final process is the Matching process where it uses the shape, size, teeth contour, dental works and identifies the image from the database. [9, 1]

1.2 IMAGE PICTORALIZING TECHNIQUE IN DENTAL BIOMETRIC:

In dental biometric technique images are pictorialized using two techniques. They are Dental photography and Dental radiography techniques.[10] In Dental photography, it gives a pictorial view of the teeth structure and its neighboring teeth with their appearance and shape. They are done using any digital camera.

Figure 3

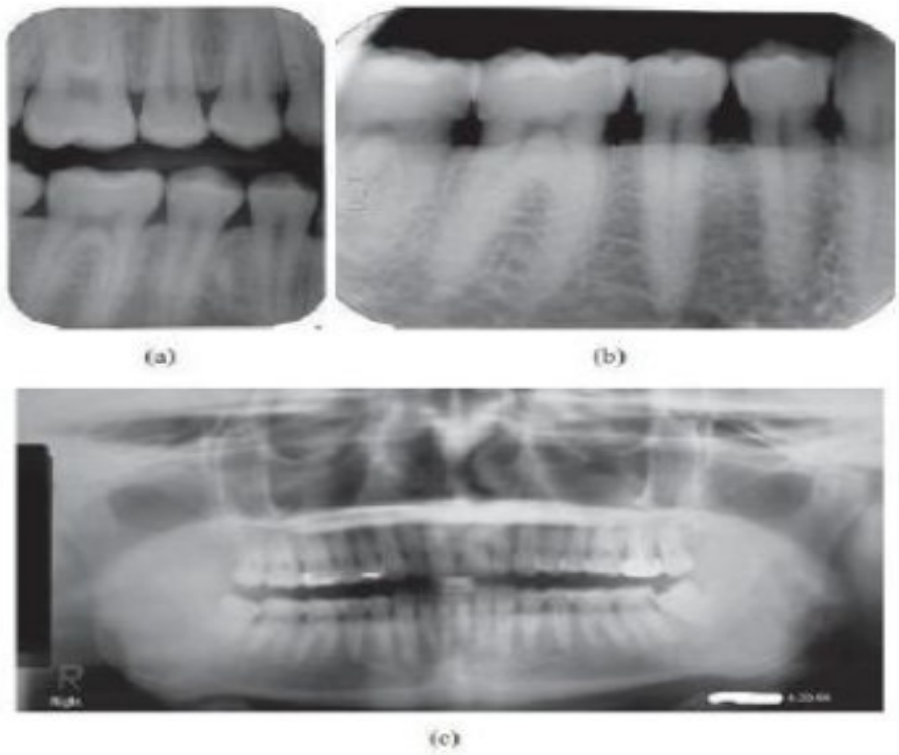
Example of Dental photography



Whereas the Dental radiography are done using X-ray radiations for intra and oral images. There are three types of Dental radiography: Periapical radiography, Bitewing radiography, panoramic radiography

Figure 4

Three types of Dental radiographs. a) Bitewing radiography; b) periapical radiography; c) panoramic radiography



2. LITERATURE REVIEW:

According to the papers we have explored the feature extraction and matching dental photography and dental radiography are the main techniques used in dental biometric. Hong Chen and Anil.K Jain describes that the feature extraction stages uses anisotropic diffusion for the enhancement of the images and Gaussian model to segment the dental work. The matching stage is done according to three steps: tooth level matching, computating the image distance and subject identification.[1, 5] Devan N.Trivedi, Ashish M.Kothari, Sanjay Shah and Shingala Nikunj uses Canny algorithm for dental image matching for human identification. The Canny algorithm process uses the edge detection method. In this process firstly the noise are removed by a low pass filter. Secondly when Canny algorithm is applied the gray images are been converted into black and white

images.[2] Mohammed shammas and Rama Krishna Alla uses the colour and shade matching technique in dentistry.[3]

Shubhangi Jadhav and Revati Shriram the uses the PM and AM records and compares them with database. They uses techniques like feature extraction, segmentation and matching. In matching process the Dental code generated is compared with the database and the finally we receive the matching percentage of the AM and PM records.[4] Stephen J.Chu, Richard D.Trushkowsky, Rade D.Paravina tells us about the different techniques used to match the tooth colour. They uses Spectrophotometers, Colorimeter and imaging system for tooth colour measurement and analysis.[6] Shubhangi Dighe and Revati Shriram proposed that they uses three techniques in preprocessing stage: Image enhancement, Edge detection and Sobel operator. In these processes the Dental radiography image are converted into gray scale image and using Sobel operator the image is detected vertically and horizontally. They also uses threshold for segmentation process. Threshold is use to separate the desire work from the teeth. They produce binary images which simplified image analysis and they produce a good result for dental work.[9] Eyad Haj Said, Diaa Eldin M. Nassar and Gamal Fahmy proposed to improve the teeth segmentation using the grayscale contrasting stretching technique.[8] Swarnalatha Purushotham and Margret Anouncia proposed to reduce the amount of intensity variation between one pixel to another by using nine methods in smoothening technique.[11]

3. COMPARATIVE ANALYSIS:

TABEL 1: Comparative analysis of different technique

Serial. No	Methods used	Algorithms	Parameters	Advantages
1.	Alignment and matching	Shape registration	Tooth contours	It gives automatic method for matching of dental

				radiography
2.	Shape extraction and matching	Anisotropic diffusion and Gaussian mode for segmentation of dental work	Tooth shape	Helps in missing tooth identification cases.
3.	Manual system	Integral projection	Root, teeth shape, root contour	It is used when there are less set of data to verify
4.	Automatic system	Hierarchical chamber distance	Shape and contour	Used to identify on a large database and speed of computational is high in his process
5.	Feature extraction and matching	Scale invariant feature transform	Contour shape and edge distance	It gives better matching of data
6.	Spectrophotometer, colorimeter	Optical radiations to give 3-D images	Tooth shade and colour	It gives a more precise depiction of colour than an conventional system

7.	Edge detection method	Canny algorithm	Teeth shape, Teeth edges, Teeth contour	It produces separate measurement for gradient component in each orientation

4. CONCLUSION AND FUTURE WORK:

From this survey, we review the role of dental images in identification and different types of technique used in dental biometric. We got an analysis that the most commonly used system is the dental radiography, whereas both Dental radiography and Dental photography are used during some circumstances. By this survey we have studied and found that the radiography technique gives more feasible information when compared to photography system. This dental biometric is applicable during mass disaster. Future studies tell us that there are new techniques which are still in process for poor quality images and blur images. Future works involve these additional information to improve the reliability of person identification when comes to dental imaging. There are many advanced works which are been developed for Forensic departments and dentistry. Some of them are cropping the dental film from the dental X-ray records using segmentation technique, Matching based on distance. There are also researches going for the human identification system for dental biometrics system to be embedded in a chip. In future we are about to propose an algorithm which can be assessed on a larger database to find a particular individual using the Ante mortem and Post mortem dental records with more parameters to retrieve.

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ADSORPTION OF MALACHITE GREEN DYE FROM AQUEOUS SOLUTION USING ACTIVATED CARBON PRODUCED FROM *SESBANIA GRANDIFLORA* STEM

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ABSTRACT:

Dyes are very perilous pollutant discharged in the effluents of textile industries through dyeing and rinsing processes causing destructive effects on the workers. It is therefore indispensable to remove the dyes by using a range of techniques. In the current paper adsorption technique was engaged for removal of Malachite Green dye. Malachite Green dye is a carcinogenic dye, which comes in the effluents of textile industries during dyeing and rinsing processes. In the current work, the effect of variables such as adsorbent dose and contact time on adsorption of dye was calculated. The dosage of *Sesbania grandiflora* stem charcoal was varied from 0.2 g/L - 2.0 g/L, and contact time was assorted from 30 minute to 360 minute. Maintaining all parameters constant, with the change of dose of *Sesbania grandiflora* stem charcoal, it was found that adsorption increases from 0.2 g/L to 1.1 g/L and then it becomes constant; with the vary of contact time, the adsorption increases from 30 minute to 220 minute and then becomes constant. Using batch extraction method and *Sesbania grandiflora* stem charcoal, Malachite Green can be detached from the effluents of textile industry, which is very profitable and effective methods comparison to activated charcoal available in market.

KEYWORDS:

Sesbania grandiflora stem charcoal, Adsorption, Malachite Green, Contact time, Effluents.

INTRODUCTION:

In the midst of the different organic pollutants of aquatic ecosystems, dyes are the hefty and significant group of chemicals present in industrial waste[1]. Dyes in water have an effect on the nature of water, restraining sunlight penetration into the stream and tumbling photosynthesis reaction[2]. Most dyestuffs are intended to defy environmental conditions like light, effect of pH and microbial assault and hence their presence in waste water is unprovoked and it is therefore enviable to remove coloring materials from effluents before their expulsion into the environment, for artistic reasons and in all the more important regions where water resources are scant[3]. Waste water from textile industries contains dyes in dissolved and suspended form and poses a severe health problem because it has a high concentration of both colour and organic matter. This colour of the effluents

discharged into various water bodies affects the aquatic vegetation and causes many water born diseases[4]. It has been reported that some dyes are carcinogenic and others after transformations or humiliation concede compounds such as aromatic amines, which may be carcinogenic or otherwise toxic[5]. It has also been reported that azo dyes comprise about 60 -70% of the total dyes used in the industry. Most of the azo dyes are carcinogenic in nature and create an explicit menace to the environment, above and beyond many dyes are agreeable for biological degradation[6]. Various researchers have also reported that dyes mount up in sediments at many sites, especially at locations of wastewater discharge, which has a bang on the ecological stability in the aquatic system. Groundwater systems are also pretentious by these pollutants because of discharging from soil[4]. Considering both the volume and composition of discharged effluent, the textile, dyeing, pulp, paper and printing industries are the major polluters among the industrial sector. Pulp and textile industries devour substantial amount of water in their manufacturing processes and hence produce large amounts of wastewater. The textile industries use dyes and pigments to colour their final products, such extensive use of colour often poses problem in the form coloured waste water that oblige pre- treatment prior to its dumping into the receiving water bodies. Different processes for the removal of coloured dyes from industrial have been reported in the past such as coagulation, flocculation, ion exchange, reverse osmosis, precipitation etc. these techniques do not show considerable efficacy and monetary advantages[2]. Over the years, the adsorption process has emerged as a doable and effective substitute to most of these conventional methods of treatment, which are pretty pricey. In the latest history, it has been reported that the adsorption a physicochemical process, offers a great potential for treating effluents containing objectionable compounds and renders them secure and reusable[7]. The key advantage of adsorption process for water pollution control are low venture in terms of rate, trouble-free design, easy & economical procedure and absence of noxious detrimental substances[2,7]. Activated carbon happens to be the most repeatedly used conventional adsorbent because of its high surface area. But it is expensive and at the same time the lofty cost of regeneration and losses during regeneration made carbon black less attractive. Therefore research is on to look for cost-effective, plentiful and eco-friendly adsorbent[2,7]. Utilization of agriculture solid wastes for the treatment of wastewater could be helpful not only to environment

in solving the solid waste disposal problem but also the economy. This technique has been used by various researchers and they have used various adsorbents like fly ash and red mud[2], fly ash and soil[3], lignite coal[6] , coir pith[5], tamarind fruit shell and sun flower stalks[9], sugarcane baggase pith, coir pith, brick powder[10], simaraubha shells[11], jack fruit peel[12], pipal bark[13], orange peel[14] ,apple pomade and wheat straw[15], mixed oxide coated sand (mocs)[16] etc.

In the present investigation, charcoal made from *Sesbania grandiflora* stems, an agricultural waste which is available in abundance all over India. Measuring the changes in concentration of adsorbed solution will assess the rate and the extent of adsorption at solid solution interface. Adsorption studies were carried out by shaking 50 ml of aqueous solution of adsorbate (dye) of desired initial concentration for different agitation times, at constant temperature and constant pH using temperature controlled bath. The progress of adsorption was noted at different time intervals till the saturation was attained. After the predetermined time interval, the adsorbate was removed by centrifugation and supernant liquid was analyzed spectro-photometrically to determine the residual dyes concentration at wavelength corresponding to their maximum absorbance. *Sesbania grandiflora* stem is available in abundance all over India, so, we can prepare charcoal in abundance and with ease by activating it. We can use it as an adsorbent to remove dyes from effluents of textile industries. Use of *Sesbania grandiflora* stem as an adsorbent will be beneficial and become an alternate of activated carbon in treatment of wastewater of textile industry. It will also provide extra income to marginal farmers and landless laborers. On the other hand, it also helps in removal of waste from agriculture fields.

MATERIALS AND METHODS:

Malachite Green used for study was purchased from Loba chemicals and *Sesbania grandiflora* stems were cut into small pieces of 2-3 cm and dried for 36 h at 393 K. The unprocessed material is mixed with K_2CO_3 at an impregnation ratio of 1:1. The mixture is dehydrated in an oven at 393 K for 1 day. The samples were encumbered in a ceramic boat and taken in a tubular furnace under high purity N_2 (99.99 %) flow of 100 cm^3/min. The sample is heated to 700°C and it is maintained at that temperature for 30 minute. The reactor is subsequently cooled to room temperature under N_2 flow and the sample is frequently washed with water until

the filtrate turn into neutral. The sample is dried at 373 K for 1 day to get ready the adsorbent used for the study. The carbon is then crushed and they are sieved to particle size of 125-150 mesh and stored in plastic bottles for adsorption studies.

Adsorption studies were carried out using *Sesbania grandiflora* stem charcoal. The adsorbent dose and contact times were measured. Solutions of fixed initial concentration with fixed amount of *Sesbania grandiflora* stem charcoal at constant pH and room temperature were stirred for fixed time interval on constant temperature magnetic shaker. After that 10 ml solution was taken out and centrifuged and studied on uv-visible spectrophotometer. Absorbance is noted between 496 nm and 500 nm.

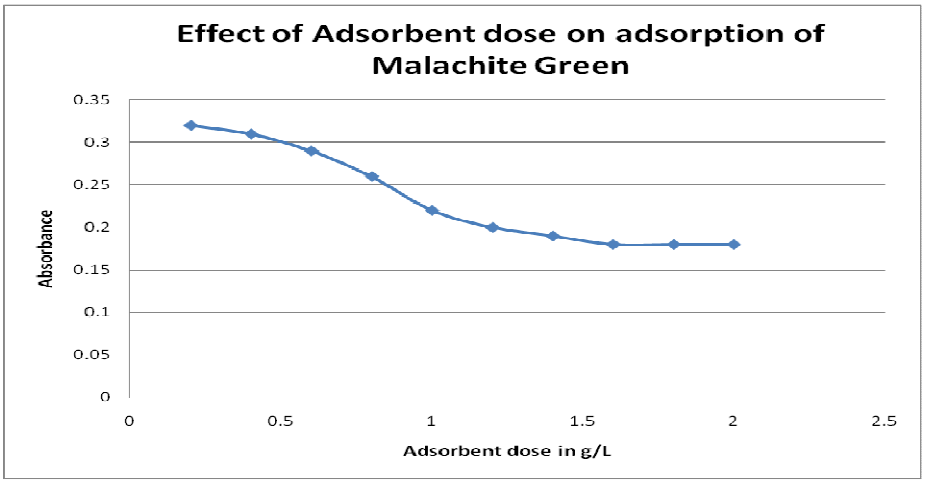
RESULTS AND DISCUSSION:

Effect of adsorbent dose:

For the study of effect of adsorbent dose, a solution of 20 ppm Malachite Green at 300^{o}C and 7.3 pH was enthused on magnetic shaker with different concentration (0.2 - 2.0 g/L) of *Sesbania grandiflora* stem charcoal for 30 min. After this, it was centrifuged and calculated on spectrophotometer. It was originated that the adsorption increased from 0.2 to 1.3 g/l dose of activated charcoal and then it became constant as shown in figure 1.

Figure 1

Effect of Adsorbent dose on adsorption of Malachite Green

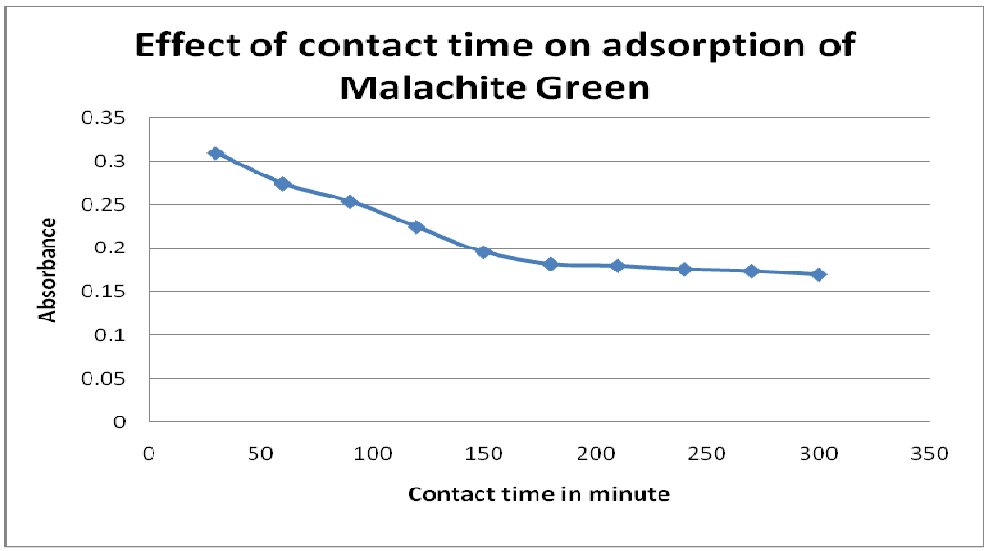


Effect of contact time:

To learn the effect of contact time, a solution of 20 ppm with 0.6 g/l activated charcoal at 7.3 pH and at room temperature was agitated for various contact time, from 30 min to 300 min. It was originated that the adsorption increased from 30 min to 200 min then it attains about constant value due to saturation as shown in figure 2.

Figure 2

Effect of contact time on adsorption of Malachite Green



CONCLUSION:

Adsorption of Malachite Green on Sesbania grandiflora stem charcoal is spontaneous process. By optimizing both factors/parameters, we can employ this trouble-free method of adsorption using batch extraction method and *Sesbania grandiflora* stem charcoal, Malachite Green can be detached from the effluents of textile industry, which is very profitable and effective methods comparison to activated charcoal available in market.

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IN VITRO EVALUATION OF THE ELECTROCHEMICAL BEHAVIOUR OF NITI SUPERELASTIC ALLOY IN SYNTHETIC URINE IN PRESENCE OF METHYLENE BLUE DYE

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ABSTRACT

NiTi shape memory alloy (SMA) as biomaterials which are used in medical implants and devices such as orthodontic wires, self expanding cardiovascular and urological stents, spine correction rods, bone fraction fixation plate and staples, and so on. The reasons for adopting NiTi SMAs in biomedical implants are their unique shape memory effects and super elasticity properties, low Young's modulus compared with stainless steels and titanium alloys, reliable and stable mechanical properties, as well as good biocompatibility. However, as are other metallic implants, when NiTi SMAs are subjected to the physiological environment, the corrosion behavior affects not only their biocompatibility but also long-term implantation safety in the human body. The aim of this paper is to study the electrochemical corrosion behaviour of NiTi superelastic alloy in synthetic urine in presence of Methylene blue dye. The scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDAX) were carried out to characterise the surface morphology and also to understand the nature of protective coating formed on the substrates. The corrosion behaviour of NiTi superelastic alloy in synthetic urine in presence of Methylene blue dye was evaluated using polarisation and impedance spectroscopy studies. The results reveal that the NiTi superelastic alloy exhibits a higher corrosion resistance in synthetic urine in presence of Methylene blue dye than in the absence of Methylene blue dye.

KEYWORDS

NiTi superelastic alloy, Methylene blue dye, Synthetic urine, SEM and EDAX, Electrochemical impedance spectroscopy.

INTRODUCTION

A large number of materials are continuously being developed to meet the requirements for different engineering applications including biomedical area. However, development of a material in this field is a challenging issue especially for those devices that are implanted in the human body, because the material must fulfill an array of fundamental biological and mechanical requirements. Among these, orthopedic applications require careful attention as a result of ageing population worldwide, large number of injuries and the demand for higher quality of life. A wide range of materials including metals, alloys, ceramics, polymers and composites are currently used in this area, but unfortunately, some have shown tendencies to

cause device failure after long term use in the body since they cannot fulfill some vital requirements.[1,2] Nowadays, shape memory alloys (SMA), and in particular nickel-titanium alloys (NiTi), is commonly used in biomedical applications.[3,4] The main attractive features of this class of materials are the capabilities of: recovering the original shape after large deformations induced by mechanical load (pseudo elasticity) and maintaining a deformed shape up to heat induced recovery of the original shape (shape memory effect). Shape memory alloys (SMA) have provided new insights into biomedical area for cardiovascular, orthopedic and dental applications, and for making advanced surgical instruments. The biomedical success of these materials is due to their unusual properties, which makes them superior to conventional materials. Among many SMAs, NiTi alloy is considered to be the best because of its superb characteristics. NiTi alloy possesses most of the necessities for orthopedic implantation and is used in a large number of applications. Therefore, it is worth to highlight the orthopedic applications of this material. NiTi alloy is quite new in medical use. It provides possibilities to make applications that no other implant material has offered before. A few commercial applications have been successfully developed since the 1970s, when Nitinol was first reportedly used for medical purposes. These applications include dental arch wire, vena cava filter and suture anchor for orthopedic surgery. In the 1990s, further development has been carried out with markedly increasing interest. Urethral, esophageal and intracoronal stents, aneurysm prostheses, and some orthopedic implants seem promising.

The present work is undertaken i) to evaluate the corrosion inhibition efficiency of NiTi superelastic alloy in synthetic urine in presence and absence of methylene blue dye. Ii) To analyse the protective film formed on NiTi superelastic alloy by SEM and AFM techniques.

MATERIALS AND METHODS

MATERIALS:

Nickel Titanium super elastic alloy was chosen for the present study. The composition of Ni-Ti super elastic alloy was (wt %) Ni 55.5, and balance Ti.[5] The metal specimens were encapsulated in Teflon. The surface area of the exposed metal surface was 0.0785 cm^2.The metal specimens were polished to mirror finish and degreased with trichloroethylene. The metal specimens were immersed in synthetic urine (SU) (J.

Przondziono et al, 2009) [6], whose composition was: Solution A: $CaCl_2.H_2O$-1.765g/l,Na_2SO_4 - 4.862g/l, $MgSO_4.7H_2O$ - 1.462g/l, NH_4Cl - 4.643g/l, KCl – 12.130g/l. Solution B: $NaH_2PO_4.2H_2O$ - 2.660g/l, Na_2HPO_4 - 0.869 g/l, $C_6H_5Na_3O_7.2H_2O$ - 1.168 g/l, NaCl - 13.545 g/l. The pH of the solution was 6.5 (W.Kajzer et al, 2006).[7]

In electrochemical studies the metal specimens were used as working electrodes. Synthetic urine (SU) was used as the electrolyte (10 ml). The temperature was maintained at 37 ± 0.1^0C.Commercially available methylene blue dye (MBD) was used in this study.

METHODS:

Potentiodynamic polarization study:

This study was carried out using a CHI 660A electrochemical impedance analyzer model. A three - electrode cell assembly was used. The working electrode used was NiTi Super elastic alloy with 1 cm^2 exposed area. A saturated calomel electrode (SCE) was used as reference electrode. A rectangular platinum foil was used as the counter electrode. Polarization curves were recorded after doing iR compensation. The parameters such as Tafel slopes, Corrosion current (I_{corr}) and Corrosion potential (E_{corr}) were calculated.

Scanning electron microscopic studies (SEM):

The NiTi Super elastic alloy specimen immersed in synthetic urine solution for a period of one day was removed, rinsed with double-distilled water, dried, and observed in a scanning electron microscope to examine the surface morphology. The surface morphology measurements of the metals were examined using JOEL-6390 computer-controlled scanning electron microscope instrument.

RESULTS

Analysis of polarization curves:

The Potentiodynamic polarization curves of NiTi Super elastic immersed in various test solutions are shown in Fig.1 (a), (b) and (c). The corrosion parameters, namely corrosion potential (Ecorr), Tafel slopes (b_c = cathodic; b_a = anodic), linear polarization resistance (LPR) and corrosion current (I_{corr}), are given in Table 1. The changes were observed in the polarization curves after addition of the inhibitor are usually used as criteria to classify inhibitors as cathodic, anodic or mixed (Bethencourt et al).[8] From Figure 1, it can be seen that the anodic and cathodic current densities obtained in the presence of inhibitor are lower than as

compared to that of in the absence of inhibitor. The corrosion potential (Ecorr) values in the presence of inhibitor are shifted to negative direction and leftward displacement in the cathodic branch of the curves.

From these data observed that the corrosion resistance of NiTi super elastic alloy in SU increases in the presence of MBD and the corrosion potential shifts to cathodic side (more negative) in the presence of MBD. Hence it is concluded that in presence of MBD, the cathodic reaction is controlled predominantly.

Table: 1

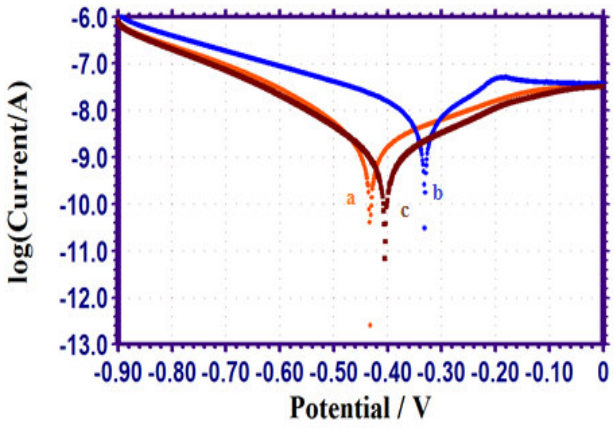
Corrosion parameters of NiTi super elastic alloy immersed in SU in absence and presence of MBD obtained by polarization study.

System	E_{corr} mV vs SCE	b_c mV/decade	ba mV/decade	LPR ohmcm^2	I_{corr} A/cm^2
SU	-0.432	124	208	1.84 x 10^7	1.84 x 10^{-9}
SU + 50 ppm Methylene blue dye	-0.331	195	127	4.42 x 10^6	7.56 x 10^{-9}
SU + 100 ppm Methylene blue dye	-0.405	128	174	3.24 x 10^7	9.94 x 10^{-8}

Figure1

Polarization curves of NiTi Super elastic in various test solutions.

a) SU b) SU+ 50 ppm of MBD c) SU+ 100 ppm of MBD



SEM Analysis of Metal Surface:

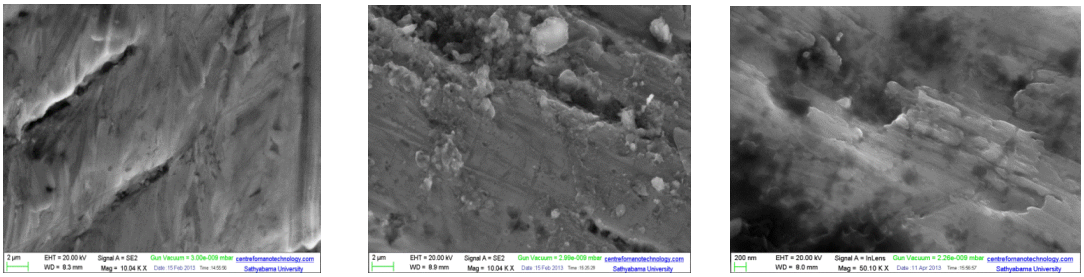
SEM provides a pictorial representation of the surface. To understand the nature of the surface film in the absence and the presence of additives and the extent of corrosion of NiTi superelastic alloy the SEM micrographs of the surface are examined. [9-11].The SEM images of of NiTi superelastic alloy specimen immersed in SU for one day in the absence and presence of additives system are shown in Figure.2. The SEM micrographs of polished NiTi super elastic alloy (control) shown in Figure.2a images illustrate the smooth surface of the metal. These show the absence of any corrosion products formed on the metal surface. The SEM micrographs of NiTi super elastic alloy immersed in SU in Figure.2b shows the roughness of the metal surface which indicates the corrosion of NiTi super elastic alloy in SU. Figure.2c indicates that in the presence of 100 ppm of MBD in SU, the surface coverage increases which in turn results in the formation of insoluble complex on the metal surface covered by a thin layer of inhibition which effectively controls the dissolution of the NiTi super elastic alloy.

Analysis of Energy Dispersive Analysis of X-rays (EDAX):

EDAX spectra were used to determine the elements present on the NiTi super elastic alloy surface before and after exposure to the additive solution [12-14]. The objective of this section is to confirm, the results obtained from chemical and electrochemical measurements, when a protective surface film of additive is formed on the metal surface. To achieve this goal, EDAX examinations of the metal surface were performed in the absence and presence of an additive system.

The energy dispersive spectroscopy (EDS) of NiTi super elastic alloy specimen polished is shown in Figure 3a. This indicates the presence of Nickel (Ni), Titanium (Ti), Carbon (C) and Oxygen (O) on the metal surface. Figure 3b shows the EDAX analysis of NiTi super elastic alloy surface immersed in SU. The analysis shows the presence of characteristic peaks of corrosion product elements (Ti, Ni, Fe, O, C and V). Figure.3c represents the EDAX analysis of NiTi super elastic alloy immersed in SU containing 100 ppm of MBD. The analysis shows the formation a protective film on the metal surface. The surface of the NiTi super elastic alloy is preserved to a large extent due to formation of the protective film of the additive molecule as indicated by the increase of Titanium peak and decreases of Ni peak in Figure .3c.

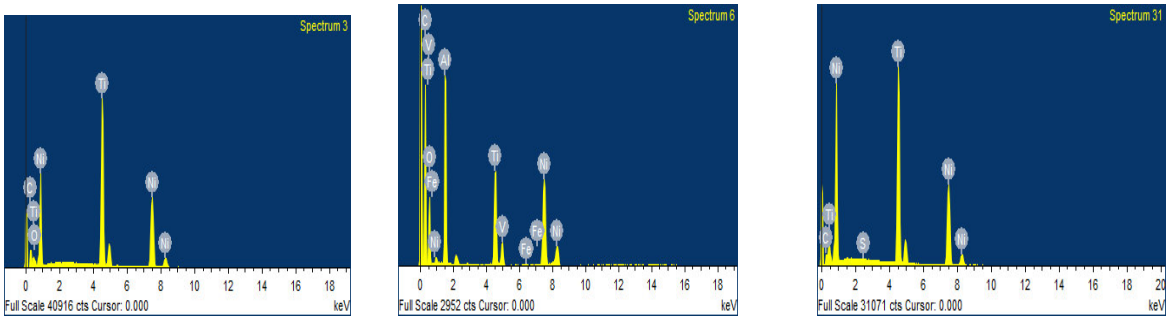
The appearance of these peaks are the notable decrease in Ni peak in the presence of an additive indicated that the protective film formed is strongly adhered to the surface, leading to a high degree of IE. This result suggests that MBD is coordinated with $Ni^{2+}$and Ti^{2+}, resulting in the formation of complex on the anodic sites of the metal surface and some of the compounds are precipitated on the cathodic sites of the metal surface. The intensity is decreases due to the formation of film coated on the metal surface.



(a) **(b)** **(c)**

Figure 2

SEM images of NiTi super elastic alloy a) Polished NiTi super elastic alloy b) NiTi super elastic alloy immersed in SU c) NiTi super elastic alloy immersed in SU containing MBD



(a) **(b)** **(c)**

Figure 3

EDAX images of a) Polished NiTi super elastic alloy b) NiTi super elastic alloy immersed in SU c) NiTi super elastic alloy immersed in SU containing MBD

CONCLUSION

The present study leads to the following conclusion, Polarization study reveals that NiTi super elastic alloy is more corrosion resistance in SU containing MBD than SU. The SEM micrographs studies confirm the formation of thin protective layer on the metal surface in SU in presence of MBD and prevent the corrosion.

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GC-MS ANALYSIS OF METHANOLIC EXTRACT OF *TECOMA STANS*

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ABSTRACT

The genus *Tecoma* is a native of Central and South Africa and they are traditional known for its therapeutic and religious purposes .The methanolic extracts of various parts (leaf, stem, flower) of *Tecoma stans* were subjected to GC-MS analysis during the period of study. The stem and flower extracts recorded eight phytochemicals and six in the leaf extract. The common phytochemical registered in all the samples were pentadecanoic acid with retention time ranging from 17.07 to 17.17. The leaf extract registered phytol which is known for its high therapeutic evidences.

KEYWORDS

Methanol, Phytochemicals, Therapeutic, Retention time

INTRODUCTION:

Traditional medicines stresses the use of plant metabolites as medicines for treating many diseases [1]. The Chinese have well established the medical field based on the phytochemicals and actively participating in the export and import of medicines to various part of the world [2,3]. Traditional medicines known as the complementary or an alternative medicine are used to prevent diagnose , improve or treat various human illness[4,5.] Medicinal plants used for evident of high therapeutic potential and believed to be a high novel drug[6,7]. Plants are known for rich source of primary and secondary metabolite which are evident as effective chemotherapeutic because of a variety of structural arrangements and properties[8-11.]

In the present investigation , the phytochemicals constituents of *Tecoma stans* were analysed by GC-MS . The significant of GC-MS is a combination of mass spectroscopy and separation gives a thorough validation of the phytochemicals[12] and also proves to be a valuable method to analysis non polar components and volatile essential oils, fatty acids and lipids[13,14]. Many screening research for phytochemicals were carried out in the various parts of the plant *Cassia italica*[7],*Nervilia aragoana*[1],Vernonia cinerea[15],*Stylosanthes fruticosa*[16], *Tagetes erecta*[17], *Acacia nilotica*[18], *Ceropegia*

pusilla[19]etc. The efficacy of the phytochemicals depend on the biological potency and their role against various diseases , including cancer[20]and Alzhemir's disease[21].

MATERIALS AND METHODS:

Tecoma stans is a species of flowering perennial shrub in the trumpet vine family, *Tecoma stans* is the official flower of the United States Virgin Islands and the floral emblem of the Bahamas. Yellow trumpetbush is an attractive plant that is cultivated as an ornamental. It has sharply toothed, lance-shaped green leaves and bears large, showy, bright golden yellow trumpet-shaped flowers. It is drought-tolerant and grows well in warm climates. The flowers attract bees, butterflies, and hummingbirds. The plant produces pods containing yellow seeds with papery wings. The plant is desirable fodder when it grows in fields grazed by livestock. yellow trumpetbush is a ruderal species, readily colonizing disturbed, rocky, sandy, and cleared land and occasionally becoming an invasive weed.

Kingdom: Plantae, unranked: Angiosperms, unranked: Eudicots, unranked: Asterids, Order: Lamiales, Family: Bignoniaceae, Genus: Tecoma, Species:T stans .

The whole diseased free plant were segregated into leaf, stem and flower after thorough washing in tap water. The various parts were air dried , powdered and stored in air tight containers separetly for further investigations. The known amount of the powdered leaf, stem and flower samples were subjected for extraction with methanol solvents. The methanolic extracts (leaf, stem and flower) were investigated for phytochemical screening by GC-MS as per standard methods.

RESULTS AND DISCUSSION:

Methanolic extract of leaf , stem and flower of *Tecoma stans* were subjected to GC-MS analysis. In the present study, the leaf registered six different chemicals and the stem and flower registered eight phytochemical each. The leaf sample registered six phytochemicals namely 11-dodecenoic acid, hexadecanoic acid, propanedioic acid, pentadecanoic acid, elcoganoic acid and phytol. The maximum retention time recorded was with elcoganoic acid with 151 ions and the minimum retention time was with propanedioic acid (10.28) with 143 ions (Table 1). The maximum ions (166) was recorded in phytol and the minimum ions in 11-dodecenoic acid (92) ions . GC-MS analysis of p.glabrum revealed 10 phytochemicals

and they are highly used as herbal alternative and eefective antimicrobial agent[22] and around twenty chemical constituents from Vernonia cinerea[15].

TABLE-1

GC-MS RESULTS OF METHANOLIC LEAF EXTRACT OF *TECOMA STANS*

s.no	Phytochemicals	retention time	no.of ions
1	11-dodeecenoic acid	12.28	92
2	Hexadecanoic acid	18.2	74
3	Propanedioic acid	10.28	143
4	Pentadecanoic acid	17.07	104
5	Elcosanoic acid	20.03	151
6	Phytol	18.97	166

In the present study ,around eight phytochemical constituents were identified in both methanolic extract of stem and flower, respectively. The maximum retention time was observed with morin with 268 ions, followed by 18.13 retention time in mitoflaxone with 152 ions (Table 2). The other phytochemicals were 4,7 octadecadeinoic acid (19 rt), terahydrotecomanica (10.23 rt),pentadecconoic acid(17.17 rt),propanedioic acid(10.65 rt),cycloisolongisolena (12.03 rt) and 1,3-cyclohexanedic acetic acid (12.27 rt). The entadeconoic acid was found to be in all three samples with retention time ranging from 17.07- 17.17 during the analysis . The rhizome of Nervilia arogoana showed a significant member of phytochemicals with ascorbic acid as a predominant constituents.

TABLE-2

GC-MS RESULTS OF METHANOLIC STEM EXTRACT OF *TECOMA STANS*

s.no	Phytochemicals	retention time	no.of ions
1	Mitoflaxane	18.13	152
2	4,7 octadecadeinoic acid	19	236
3	Morin	19.95	268

4	Tetrahydrotecomanine	10.23	88
5	Pentadecanoic acid	17.17	193
6	Propanedioic acid	10.65	143
7	Cycloisolongifolene	12.03	141
8	1.3-cyclohexanedi acetic acid	12.27	92

The first photochemical registered in the methanolic flower extract was pentadecenoic acid with 17.12 retention time of 104 ions within it. The other phytochemicals registered in the sample were 10-octodeonoic acid (18.85 retention time),tridecacoic acid (7.77 retention time),benzeneacetic acid (14.12 retention time), 1,7- dinitrophenazine5-oxide (15.1 retention time), 2 ethylne dioxyl ethylamine (10.25 retention time), 1,4-dioxacycle hexadecane-5 (18.13 retention time), and penta 1,4-dien-3-one (19.95 retention time). The minimum ions registered was with 74 ions in benzene acetic acid (Table 3) and the maximum was observed in 10-octodeonoic acid with 249 ions (Table 3).

TABLE-3

GC-MS RESULTS OF METHANOLIC FLOWER EXTRACT OF *TECOMA STANS*

S.No	**Phytochemicals**	**Retention Time**	**No. of Ions**
1	Pentadeconoic acid	17.12	104
2	10-octodeonoic acid	18.85	249
3	Tridecanoic acid	7.77	177
4	Benzeneacetic acid	14.12	74
5	1,7-dinitrophenazine5-oxide	15.1	159
6	2-ethylamine	10.25	215
7	1,4-dioxacyclohexadecane-5	18.13	148
8	Penta-1,4-dien-3-one	19.95	167

CONCLUSION:

GC-MS analysis of *Tecoma stans* plant proved to contain various types of phytochemicals which have high therapeutic value in the traditional medicines. Further investigations on these extracts will provide a detail aacount on their efficient role in the various sectors of curing diseases and it will venture into a combinations of developing a novel drug in near futures.

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PHYTOCHEMICAL SCREENING STUDIES OF *SPAHGNETICOLA TRILOBATA*

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ABSTRACT:

Around 6000-7000 flowering plants are recorded to have medicinal usage in Ayurveda, Siddha, Unani and Homeopathy. *Sphagneticola trilobata* belongs to family Asteraceae with genera Aster family and they are from Mexico, Central America and Caribbean region. In the present study *Sphagneticola trilobata* plants were segregated into various parts (Leaf, Stem and Flower) and subjected to qualitative phytochemical screening with methanol as solvent. Around15 phytochemicals parameters were carried out during the present study and it was proved that the leaf, registered the maximum number (11) of phytochemicals than stem and flower samples.

KEY WORDS:

Therapeutic, Phytochemicals, Extract, Alkaloids, Terpenes, Anti – inflammatory.

INTRODUCTION:

The study of traditional human utility of phytochemicals (Ethnobotany) has been recognized as an effective and efficient way for development of new drugs for near and future. About 12,000 chemical compounds were isolated so far and out of which 10% of the total compounds are employed as potential conventional pharmaceutical drugs[1,2]. A survey on 2001 revealed that 122 phyto compounds were used in modern medicine (eg. Aspirin, Digoxin and Opium)[3]. World Health Organisation (WHO) estimated that 80% of the Asian and African population uses as herbal medicines in terms of primary health care[4]. In India, Ayurvedic medicine, uses many phytochemicals from 1900 BC[5,6] and there are documentations from and Rig veda and Atharvaveda about the use of herbs as medicine[7]. In the 6th century BC (Suhruta Samhita) evidented around 700 medicinal plants and 57 medicinal combinations from animal sources[8]. The therapeutic values of the phytochemicals such as Tannins, Flavanoids, Phenols, Alkaloids have a definite physiological on various aliments of human body[9,10]. Almost all the phytochemicals are anti inflammatory[11] anticonstipative[12], antiinsecticidal[13], antiplasmodial[14], antifungal[15], antioxidant[16] etc. Phytoscreening and antioxidant studies of *Ocium sanctum, Menthaspicata ,Trigonella forum* graecum proved to be highly

therapeutic and employed in various commercial drug[17,18] .In the present investigation, an attempt has been made to screen for the presence of phytochemicals from *Sphagneticola trilobata* plants.

MATERIALS AND METHODS:

The whole plants (Disease free) were collected, segregated into leaves, stem and flower, air dried, powdered and stored in a air tight container for future investigations. In the present study the powdered plant parts (Leaves, Stem and Flower) were subjected to various qualitative phytochemical analysis as per standard methods such as Carbohydrates[19], Tannins[20], Saponins[21], Flavanoids, Cardiac glycerides, Terpenoids, phlobatanins, anthraquinones [22] , Alkaloids [23] , Quinones , Phenols and Coumarins[24] . A known quantity of the leaf , stem and flower sample were incubated in methanol solvent and the filtered methanolic extract were used for investigation.

RESULTS AND DISCUSSION:

The powdered samples of leaf, stem and flower of *Sphagneticola trilobata* were subjected to various photochemical analysis as per standard methods. The results obtained were tabulated in the Table 1 and 2. Around 15 phytochemical analysis were carried out during the period of study and the results proved to have high amount of phyochemicals. The leaf sample of *Sphagneticola trilobata* sample registered a distinct positive result for carbohydrates, tannins, saponins, alkaloids, quinones, glycosides, terpenoids, phenols, proteins , phylobatanins and steroids and polysteroids (Table 1 and 2).

Table 1

Qualitative Phytochemical Analysis of methanolic extract of Sphagneticola *trilobata*

S.NO	PHYTOCHEMICALS	METHANOLIC EXTRACT		
		LEAF	STEM	FLOWER
1.	Test for Carbohydrates	+	+	+
2.	Test for Tannins	+	+	+
3.	Test for saponins	+	+	-
4.	Test for Flavanoids	-	+	-

5.	Test for alkaloids	+	-	-
6.	Test for quinones	+	+	+
7.	Test for glycosides	-	-	-
8.	Test for cardiac glycerides	+	+	+
9.	Test for Terpenoids	+	+	+
10.	Test for Phenols	+	+	+
11.	Test for Coumarins	-	+	-
12.	Test for Proteins and amino acids	+	+	+
13.	Test for Steroids	+	+	+
14.	Test for Phylobatanins	+	-	-
15.	Test for Anthraquinones	-	-	+

The methanolic extract of the stem registered 11 positive results out of 15 photochemical analysis. The stem of *Sphagneticola trilobata* did not registered positive result for alkaloids, glycosides, phylobatanins and anthraquinones. The flower extract showed eight positive results for carbohydrates, tannins, quinones, cardiac glycerides, terpenoids, phenols, proteins and amino acids and anthraquinones and seven negative results for saponins, flavanoids, alkaloids, glycosides, coumarins, steroids and phylobatanins (Table 1 and 2).

Table 2

Results of Phytochemical screening of Methanolic Leaf extract of *Sphagneticola trilobata*

S.NO	**PHYTOCHEMICAL TESTS**	**OBSERVATION OF METHANOLIC EXTRACT**		
		LEAF	STEM	FLOWER
1.	Test for Carbohydrates	Reddish colour	Reddish colour	Reddish colour

2.	Test for Tannins	Dark blue or greenish colour	Dark blue or greenish colour	Dark blue or greenish colour
3.	Test for saponins	Layer of foam	Layer of foam	No layer of foam
4.	Test for Flavanoids	No yellow colour	Yellow colour	No yellow colour
5.	Test for alkaloids	Green colour or white precipitate	No green colour or white precipitate	No green colour or white precipitate.
6.	Test for quinines	Red colour	Red colour	Red colour
7.	Test for glycosides	No pink colour	No pink colour	No pink colour
8.	Test for cardiac glycerides	Brown ring	Brown ring	Brown ring
9.	Test for Terpenoids	Red brown colour	Red brown colour	Red brown colour
10.	Test for Phenols	Blue or green colour	Blue or green colour	Blue or green colour
11.	Test for Coumarins	No yellow colour	Yellow colour	No yellow colour
12.	Test for Proteins and amino acids	Blue colour	Blue colour	Blue colour
13.	Test for Steroids	Brown ring	Brown ring	No brown ring
14.	Test for Phylobatanins	Red colour	No red colour	No red colour
15.	Test for Anthraquinones	No pink	Pink colour	Pink colour

		colour		

From the above investigation, it was found that the Leaf, Stem and flower of *Sphagneticola trilobata* showed positive results for carbohydrates (Red colour), tannins (Dark blue or greenish colour), saponins (Layer of foam), cardiac glycerides (Brown ring), terpenoids (Red brown colour), phenols (blue or green colour), proteins and amino acids (Blue colour). Similar results were observed with both ethanol and petroleum ether for *Tagetes erecta*[10] and *Artemisia milagirica*[18] (Methanol, Ethanol and Petroleum ether). The leaves of Cleome rutidosperma also registered alkaloids, tannins, saponins, flavanoids and cardiac glycerides[25.] The leaves of *Euprobia reterophylla* also registered the presence of alkaloids, phenols, tannins, flavanoids[26].

CONCLUSION:

The present investigation has proved that *Sphagneticola trilobata* contains secondary metabolites like saponins, flavanoids, tannins, cardiac glycerides, terpenoids , phenols and proteins and amino acids in all the parts of the plants and it also reveals that the plant has high therapeutic value. On further research with various parameters, it will pay a way for a new and novel drug with less side effects.

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EFFECT OF RHIZOMES OF *WITHANIA SOMNIFERA* AGAINST ENDOSULFAN INDUCED HEPATIC DEGENERATION IN FRESH WATER *CATLA CATLA*

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ABSTRACT

Effect of aqueous extract of root of *Withania somnifera* (WSR) on the biochemical alteration in few profiles of Liver Function Test (LFT) in hepatic tissue of endosulfan induced fish was investigated. Serum Alanine Amino Transferase (ALT), Asperate Amino Transferase(AST),Alkaline Phosphatase(ALP) and Acid Phosphatase(ACP) showed a significant rise in test group (4 ppb & 8 ppb endosulfan treated). The animals were subjected to aqueous WSR extract @ 50 mg/kg body weight) for 14 days by gavage method. A significant decrease in ALT, AST, ALP and ACP level in curative group (WSR treatment in endosulfan exposed group) was observed. Biochemical highlight the modulatory effect of aqueous WSR extract against endosulfan induced hepatic injury in fish.

KEY WORDS

Endosulfan, *Withania somnifera, CATLA CATLA*, ALT, AST, ALP, ACP.

INTRODUCTION:

Physiochemical changes in aquatic environment drastically influence fish physiology. Endosulfan is persistent organic pesticide widely used in agriculture & horticulture fields of more than 70 countries1. Fish are able to accumulate several fold higher concentration of pesticide residue than the surrounding water (Siddiqui et al., 2005). Endosulfan is toxic to liver cells (Singh and Singh,2007). Liver and kidney is the primary organ of degradation, detoxification and elimination. These are the most affected organ by the toxic assault. The plant based formulations have been used since ancient times as remedial measures against various human and animal ailments. Plant extracts and phyto-constituents have been found effective as radical scavengers and inhibitors of lipid peroxidations (Yildirim et al., 2001). Besides, the chemical constituents present in the herbal medicines or plants are a part of physiological functions of living flora. Although phytoremediation have been used as natural antioxidant as human beings since time immemorial but its wide spectrum use in the aquatic organism is still in its infancy stage. A few plant extracts have been reported to have mitigating impact against various intrinsic diseases in aquatic organism (Siyanbolo and Ebochuo., 1993). In Ayurveda and Unani system of medicine, rhizomes of *Withania somnifera* have been used in treating various liver disorders (Akbarsha et al., 2000).

The species *Withania somnifera* (Dunal) (Family – Solanaceae) is commonly known as Ashwagandha (Hindi) or Indian Ginseng (English). The roots are stout, fleshy and whitish brown. Biochemically heterogenous rhizome alkaloids contain cuscohygrine, anahygrine, tropine, pseudotropine, anaferine, isopellatierine, nicotine and withasomnine, solasodine, visamine, dulcitol, glucosides, sitoindocides etc (Kulkarni and Dhir Ashis, 2008). The withanoloids are known to be antiinflammatory (Jayprakashan and Nair Murlidharan, 2003), powerful antioxidant (Rasool M, Varahlakshmi, 2007), hypocholestemic (Visavadiya NPM, Narasimhacharya, 2007), hepatoprotective (Saxena et al ., 2007), antitumourous (Saritha and Naidu, 2007), antimalarial (Muregi et al., 2007) as well as immunoprotective (Malick et al ., 2007). Most of the studies are focussed on the antioxidant properties of *Withania somnifera* on human beings and mammalian experimental model but the true modulatory impact of aqueous rhizome extract of the plant on aquatic animals have not been explored. The present study has been designed to investigate the probable mitigating impact of *Withania somnifera* root extract against endosulfan induced hepatotoxicity in freshwater air breathing fish *Clarias batrachus*, based on histopathological examination of liver tissues and biochemical assessment of few LFT (Liver Function Tests) profiles like Alanine amino transferase (ALT), Asperate amino transferase (AST), Alkaline phosphatase (ALP), and Acid phosphatase.

MATERIALS AND METHODS:

Experimental Animal:

Fingerlings of Catla catla in the weight range of 12±0.3 g and body length of 8.5±2 cm were obtained from Sirago aqua farm at Neringipettai, Tamil Nadu, India. They were safely brought to the laboratory and acclimatized for 30 days in a large cement tank (1000 l capacity) prior to the experiment. During the acclimatization period, fish were fed rice bran and groundnut oil cake in the form of dough once daily. Water was renewed (one third of the water) daily and feeding was withheld 24 h before the commencement of the experiment. In the present study tap water free from chlorine was used and the water had the following physicochemical characteristics; temperature (27.5±2 ◦C), pH (7.2), dissolved oxygen (6.4mgl−1), total hardness (90mgl−1, as CaCO3), salinity (0.4±0.02 ppt).

Experimental Design:

The acclimated fish were categorized into following 6 groups consisting of 6 fish each

Group I (Control group): Normal

Group II (WSR extract treated group): The aqueous root extract was given @ 50mg/kg b.w. for 14 days.

Group III (Endosulfan test group): 4 ppb and 8 ppb of endosulfan treated for 14 days.

Group IV (Prophylactic group): 4 ppb and 8 ppb of endosulfan for 14 days with simultaneous treatment of aqueous root extract of *W. somnifera* @ 50 mg/kg body weight for 14 days.

Group V (Curative group): 4 ppb and 8 ppb of endosulfan were given for 14 days followed by administration of aqueous root extract of *W. somnifera* @ 50mg/kg body weight for 14 days.

Group VI (Self healing group): 4 ppb and 8 ppb endosulfan treated group left without further treatment for next 14 days.

Chemicals:

Fresh root of *W. somnifera* were procured from trichy garden. The 96 hrs LC50 of endosulfan was calculated by standard APHA,(2005), method and confirmed by pilot test as 20 ppb. The fish were exposed to non-lethal dose of 4 ppb and 8 ppb respectively. Gluteraldehyde and OSO4 were obtained from Sigma Chemicals, USA and all kits and chemicals used for estimation of ALT, AST, ALP and ACP (Reitmann and Frankel, 1957) were of reagent grade and purchased from local Merck India distributor.

Preparation of plant Extract:

Lyophilized aqueous rhizome extract of *W. somnifera* was prepared(Prabhu and Patel ,1995). The rhizomes were weighted, washed, thoroughly grinded to a paste in motors and then homogenized in Potter Elvehjen homogenizer. It is further dried in an incubator at 40℃ for 2 days, crushed in an electrical grinder and dissolved in hot distilled water. The suspension was filtered under suction and the filtrate was freeze dried using Labcono Freeze drier model 75018, yielding brown residue. The NOEL (No observed effective level) and MPD (Maximum permissible dose) of the aqueous extract of *W. somnifera* root (WSR) were

determined and the diluted WSR aqueous extract were administered by gastric intubation method daily @ 50 mg/kg b.w. for 14 days to different experimental groups.

Serum enzymes:

On the termination of exposure day blood samples were collected in a heparinised syringe from cardiac puncture. The serum was separated by centrifuging at 5000 rpm for 10 minutes at 4℃. The serum was assessed for ALT, AST, ALP and ACP.

Statistical analysis:

The data obtained in each group were analyzed using students' 't' test. Values of $p<0.05$, $p< 0.01$ and $p<0.001$ were considered to be significant. All the statistical analysis were done using sigma plot 8.0 version.

RESULTS:

The extent of liver damage was assessed by estimating serum level of ALT, AST, ALP and ACP (Table – 1, 2). The test group (group III) showed a significant ($p<0.01$) increase in ALT, AST, ALP and ACP levels in both 4 ppb and 8 ppb endosulfan exposure. However, the percentage increase was higher in 8 ppb exposure. The prophylactic group (Group IV) showed increasing trend in all the enzymes when compared to control group (Group I) but showed a characteristic significant decline in comparison to test group. The curative group maintained for 14 days showed an improvement in the liver functioning as reflected by significant ($p<0.01$) decreased enzyme level. Only WSR extract treated group (Group II) showed a non-significant decline in serum level of ALT and AST, while a non significant increase in serum level of ALP and ACP, when compared to control. Self healing group (Group VI) showed a non-significant decline in enzyme level in contrast to test group.

Table – 1

Assessment of liver damage in various groups at 4 ppb endosulfan exposure.

Parameters /unit	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI
ALT(U/ml)	28.56±2.74	30.66± 2.946	54.06± 5.20	42.84± 4.12	38.76 ±3.73	53.04 ± 5.10

AST(U/ml)	14.280± 1.375	12.240± 1.178	30.600± 2.946	23.460± 2.258	17.340± 1.669	30.600± 2.946
ALP(KA units)	3.060±0. 295	3.998±0. 385	14.790± 1.424	8.466± 0.815	7.650± 0.736	15.504± 1.492
ACP(KA units)	0.510±0. 049	0.745±0. 072	3.019± 0291	2.040± 0.196	2.020± 0.194	3.876± 0.373

Values are expressed in Mean±SD of six replicates in each group.

Table – 2

Assessment of liver damage in various groups at 8 ppb endosulfan exposure.

Parameters/unit	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI
ALT(U/ml)	28.56±2.74	30.66± 2.946	69.360±6.676	48.960± 4.713	40.800± 3.927	58.140± 5.596
AST(U/ml)	14.280±1.375	12.240±1.178	35.700±3.436	27.540± 2.651	20.400± 1.964	34.680± 3.338
ALP(KA units)	3.060±0.295	3.998±0.385	9.180± 0.884	6.936± 0.668	5.916± 0.569	10.200± 0.982
ACP(KA units)	0.510±0.049	0.745±0.072	3.060± 0.295	1.938± 0.187	2.040± 0.96	3.876± 0.373

Values are expressed in Mean±SD of six replicates in each group.

DISCUSSION:

Hepatoprotective activity of *Withania somnifera* was explored by evaluating its effect on serum level of ALT, AST, ALP and ACP as well as histopathology of liver tissues of endosulfan treated fish. Hepatic system is the major organ system involved in the metabolism, detoxification and excretion of various endogenous and exogenously ingested substances like xenobiotics, pollutants etc. These substances alter the hepatic metabolism resulting in the generation of highly reactive free radicals which covalently bound with the membrane, alter their permeability and cause extensive tissue damage.

The hepatic cells are consistently attacked by the free radicals and cell necrosis results. Although inbuilt antioxidant system protect the tissues from free radical attack but the excessive releases of ROS results in considerable organ damage. Administration of various antioxidants to strengthen the inbuilt protective mechanism may be useful in protecting the organ against various toxicants. The sensitivity of aquatic animals to endosulfan has been explored (Glover et al, 2007).

Toxicity is known to be primarily mediated by inhibition of important ion transport proteins in a variety of tissue (Naqvi and Vaishnavi,1993) or by inducing oxidative stress (Droval et al, 2003). Administration of endosulfan as both lower (4 ppb) and higher (8 ppb) non- lethal exposure enhanced the serum biochemical markers viz. ALT, AST, ALP and ACP. At 4 ppb endosulfan exposure serum ALT showed a significant ($p<0.05$) increase of 86.66% while prophylactic and curative group showed a significant decline of 17.85% and 28.57% respectively. A non-significant recovery in serum ALT was recorded in self healing group (Table – 1). Serum ALT followed the same trend at higher sublethal exposure of endosulfan, where it showed a significant ($p<0.01$) increase of 112.15% and 162.5% at 4 ppb and 8 ppb endosulfan exposure respectively. Serum ALP and ACP showed significant ($p<0.001$) increase when compared to control. Similar kind of elevation in ALT and AST of *Channa gachua* (Koul et al, 2007) *and Clarias batrachus* (Mukhopadhyay and Deharai ,1980)*, Tilapia mossambica* (Rao KSP and Rao,1984) and *Channa striatus* (Sadhu and Chowdhury, 1985) due to different pesticide exposure has been reported. A similar increase in ALT and ALP levels was observed in CCl4 test group (Kamala kannan et al,.2005). Antioxidant effects of *Withania somnifera* in different mammalian group have been explored (Mishra Lakhminarain et al 2008, Hamaza et al., 2008). *Withania somnifera* root extract (@50mg/kg b.w. orally) treatment for 14 days in curative group showed significant decline in serum ALT by 28.5% and 38.88%, in serum AST by 41% and 35.7%, in serum ALP by 46% and in serum ACP by 46% and 54% respectively, when compared to test group (4 ppb and 8 ppb endosulfan exposure).

CONCLUSION:

The biochemical findings signify the restorative potential of WSR extract against endosulfan induced hepatotoxicity. The appropriate dose of WSR extract may be considered as an antidote to organochlorine toxicity.

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ASSESSMENT OF CITRIC ACID ACTIVITY ON WOUND HEALING IN DIABETIC ULCERS

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ABSTRACT

The objective of the study was to assess the condition of diabetic foot ulcer. It was assessed by standardised wound assessment scales before the citric acid wound dressing, after ciitric acid wound dressing, post assessment of diabetic foot ulcer. Citric acid is a source of vitamin c obtained from citrus fruits ,it promotes wound healing. The present study has proven that citric acid is effective in diabetic foot ulcer and also that citric acid wound dressing can be used in other surgical wound.

KEY WORD: *C*itric acid, Diabetic footulcer, Wound dressing

INTRODUCTION:

Diabetic foot is one of the complications of diabetes the end point is leg ambutation. Aggarwal 2004 stated that elevated blood sugar level damages the blood vessels causing them to thicken and weak which cause poor blood circulation.Holsten 2002 explained the experiences of non healing diabetic wound,reduced collagen synthesis impaired wound contraction and delayed epidermal migration. H.N.Gosh revealed the importance of Vit C is responsible for formation and maintenance of the integrity of the colloidal intracellular substance. Nogoba , *et al* 1998 evaluated the treatment of superficial pseudomonal infections with citric acid ,PB kulkarni,*et al* 2000 assessed the effectiveness citric acid treatment of diabetic foot.Robbin said the vitamin 'c'is the activation of prolyi and lysyl hydroxylase from inactive precursor,providing for hydroxylation of precollegen .Ruth mentioned deficiency of vitamin 'c'leads tosuppression of the rate of synthesisof collagen peptides,independent of an effect on protein hydroxylation.

Statement of the problem: A study to assess the effectiveness of citric acid in healing footulcer among patients ith diabetesmellitus

Objectives:

- To assess the condition of diabetic foot ulcer
- To apply citric acid wound dressing
- To evaluate effectiveness of the citric acid wound healing process

Null Hyphothesis:

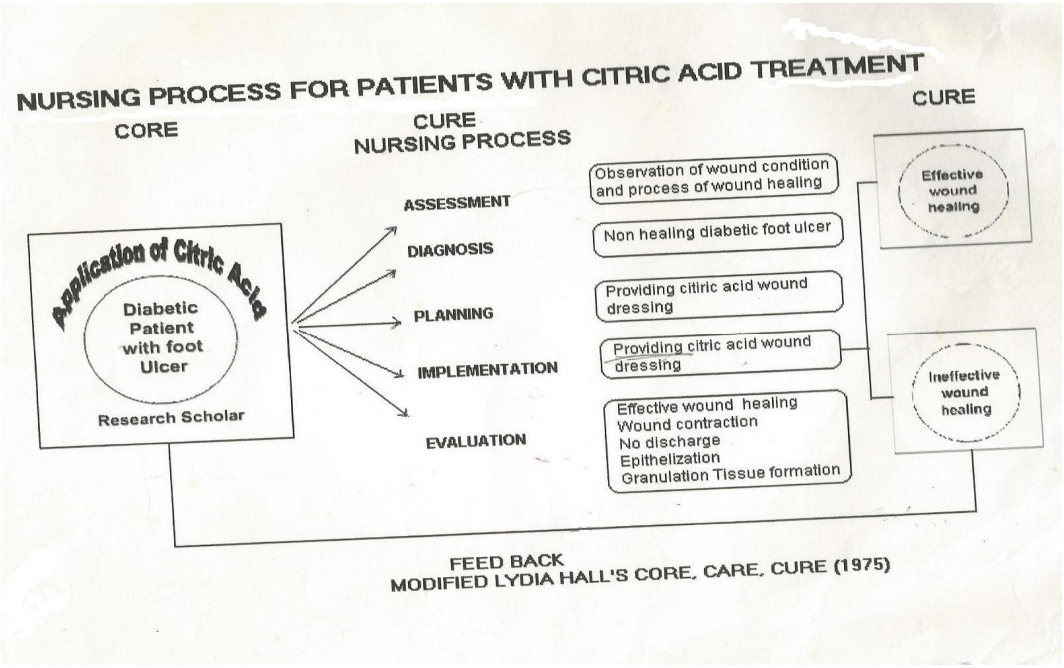
Citric acid is not effective in healing diabetic foot ulcer

MATERIALS AND METHODS:

The present study includes 40 diabetic patients with foot ulcer, the research approach adoption was quasi experimental, nonrandomized, convenient sampling technique was adopted to select the sample size. The wound was assessed by batesjenson wound assessment tool. The instrument consists of 15 observations regarding the conditions and process of wound healing the maximum score was given for condition of wound healing is 5.Commercially available Citric acid crystals were used for40 patients with diabetic foot ulcer before treating the wound with citric acid the blood sugar level was monitored and controlled.1gm of citricacid crystals was dissolved in sterile water in the ratio of 1mg/1ml.The foot ulcer treated by resection of necrotic tissue and wound irrigation by using citricacid and a guaze soaked in citric acid applied before dressing was completed. In this way dressing was done for 10 to 12 days. No antibiotic was given during the treatment of foot ulcer. Blood sugar controlled with diabetic agent only after treatment the wound assessed by standardized tool. CORE: Refers to problem of patient that is diabetic foot ulcer. CARE: Care includes observation of wound condition, providing citricacid wound dressing, assessment of wound healing after dressing. CURE: Cure refers as 12^{th} day wound were assessed by the same tool to evaluate the effectiveness of citric acid wound dressing.

Figure 1

Nursing process for patients with citric acid treatment



The collected data were computed and analysed, interpreted with the help of inferential statistics and paired't' test.

Table 1

Inferential data

S.NO	DATA ANALYSIS	METHODS	REMARKS
1.	Inferential statistics	Paired 't'test	Analyzing the effectiveness between the pre and post test result of healing diabetic foot ulcer

Demographic findings

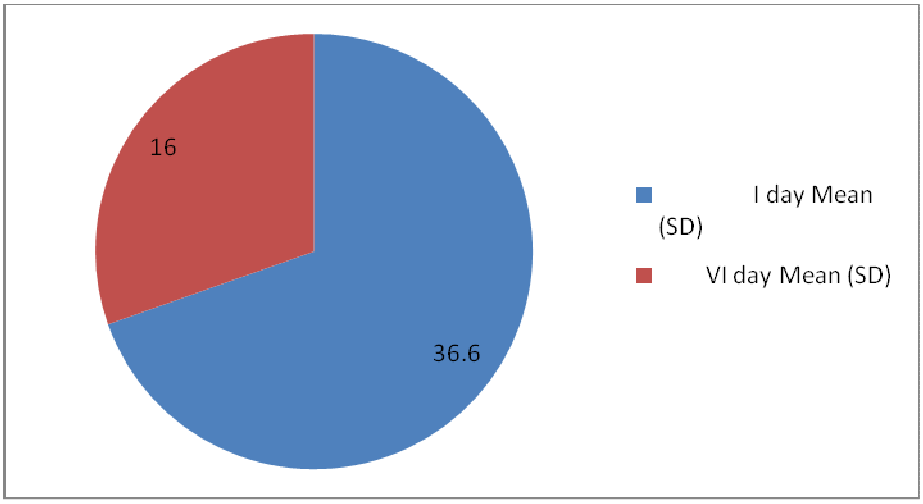
In this study majority 47.5% of diabetic foot ulcer patients were in the age group of 40-60yrs, 72.5% were males, 70% were Hindu, and 40% were middle school in experimental group. Majority 42.5% were Cooley, having the family income of more than Rs.5, 000/-. Regarding in sources of health information

majority 45% health personnel , Majority 45% in moderate working pattern. Regarding family history of diabetic 40% of patients had diabetes above 10yrs,Majority 37.5%2 -3yrs ,. Majority 32.5% had suffered from diabetes between above 5yrs.

COMPARISONS OF MEAN PRETEST AND POST TEST HEALING DIABETIC FOOT ULCER.

Figure 2

Pre test mean levels for the first day 36.6. fith day 16.0 respectively in the patient receiving citric acid wound dressing.



*** - statistically significant

Figure 3

6th day of Citric Acid Wound Healing:

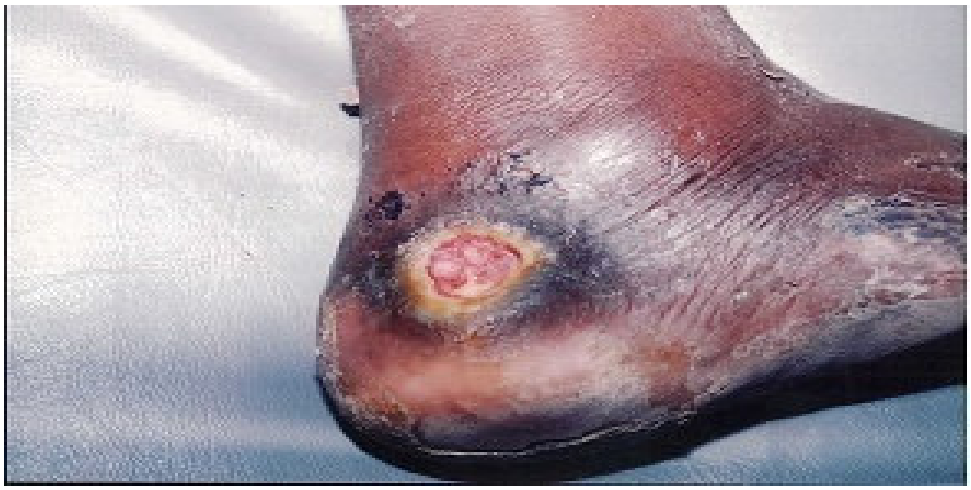
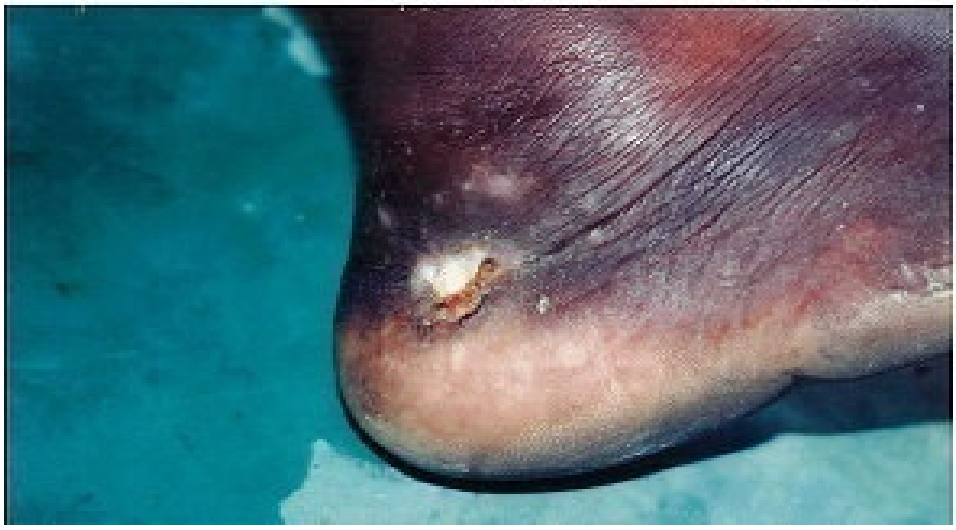


Figure 4

12th day of Citric Acid Wound Healing:



The mean post test foot ulcer level is 16.0 was lower in the experimental group. The obtained paired't' test value is 29.3 *** was statistically significance at P<0.001. It is inferred that citric acid dressing was effective in healing foot ulcer among diabetic patients.The interpretation of above findings clearly picturised that the mean values of the diabetic foot ulcer has a positive difference with't' values, statistically significant at P<0.001. This indicates that there is a significant healing foot ulcer and improvement in granulation, epithilialisation occurred after using citric acid wound dressing.

CONCLUSION:

This study shows that diabetic foot ulcer can be treated with citric acid wound. This study also confirms that this treatment does not require any other preparation except dressing the wound. Citric acid crystals are commercially available and economically affordable .Hence Citric acid is proven to be effective against diabetic foot ulcer.

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STUDIES ON THE MOULTING AND REPRODUCTIVE BIOLOGY OF THE MOLE CRAB EMERITA ASIATICA (MILNE EDWARD)

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ABSTRACT

In Crustaceans , adaptive nature shows a variety of reproductive strategies that enable them to successfully colonise in their respective habitat**.** Moult cycle is a constitutive phenomenon in Crustaceans, with molting being a complex and energy demanding process. The relationship between moulting and reproduction is more marked in females, as active vitellogenesis during the reproductive cycle, as well as secretion of a new cuticle during molting ,could affect the physiology of the organism by their competitive utilisation of reserve material from storage organs(Subramanian (2000).Reproduction and metabolism in crustaceans depend on the molt stage and all three phenomenon-molt, reproduction & metabolism are correlated with the season (Aiken,1969;Conan1985;Bouchon et al;1992).In the mole crab Emerita asiatica, the female reproductive cycle is repetitive. When the pleopodal embryos undergo development, there is a concurrent maturation of oocytes within the ovary making it ready for the next spawning. However molting occurs after hatching of the larvae from the pleopods and before spawning. Breeding cycles are correlated with seasonal changes in such a way that the off springs are produced at a time most favourable to the survival.

KEYWORDS

Moulting, Emerita asiatica, Spawning, Reproduction, Proteins

INTRODUCTION

Among arthropods, crustaceans occupy both aquatic and terrestrial habitats with tremendous adoptive diversity (Barnes, 1974**).** Therefore most aspects of the life history of crustaceans are, atleast to some extent, synchronised with the moult cycle(chang1995).Species belonging to the genus Emerita occurring in the intertidal zone, extend in distribution from tropical to temperate sea . Growth in crustaceans includes all physiological, biochemical and morphological changes that occur during the periodic shedding and reformation of new exoskeleton. In crustaceans three types of relationship exists between molting and reproduction [Adiyodi and Subramanian, 1983] In crabs, lobsters where the reproduction starts during the intermolt period. Isopods, amphipods and shrimps shows synchronization of both molting and reproduction, Cirripedes require several molt cycles to complete the reproduction**.** Interrelationship between moulting and reproduction in decapode crustaceans has been mainly studied in reference to the interplay of hormonal system controlling them (Adiyodi and Adiyodi 1970)It is well known that the reserve organ contributing to moulting and reproduction is one and the same namely hepatopancrease. Therefore a well defined

coordination for the molting and reproductive event is essential for a successful partition of energy reserve for these process. In the adult stage moulting and reproduction could not ignore the possible interference of each other. The prawn species belonging to Decapoda show short intermoult, but the premoult is a lengthy period. Thus the majority of the ovarian development might occur during the prolonged premoult period . The spawning might occur after a facultative moult as shown in Macrobrachium nobilli (Pandian and Balasundaram, 1982). In E.asiatica the developmental stages of setae on the pleopods and the extent of epidermal retraction were used to define the moult cycles stages (Gunamalai and Subramonium,2002).Crustaceans ,with high fecundity and faster body growth exhibit closeness in their molting and oogenic cycle (Gunamalai and Subramonium,2002).

METERIALS AND METHOD

Molting stages:

The mole crab (Milne Edward) used in the present investigation were collected from the Intertidal sandi shore. They occur in colonies in the intertidal belt cl.ose to the water edge and move up and down during high and low tide. As the pleopod of the crab is relatively transparent ,they were used to classify the molting stages. Molting stage is characterised by observing the level of retraction of epidermis from cuticle. The terminal part of the pleopod is cut and placed in a drop of clean sea water and examined under a compound microscope. They were taken to the laboratory and maintained in a trough with seawater and sand. In the present investigation the epidermal retraction as well as the setal formation on the pleopod and the related changes in the cuticle of the pleopod ,are used as the main criteria for distinguishing moult stage as described by Vigh and Fingerman (1985). Only female measuring 27-33mm in carapace length were used throughout the investigation.

Ovarian Stages:

For the present investigation moulting stages of Emerita asiatica was characterized using pleopodal observation as an index as described by Vigh and Fingerman (1985) for the crab Uca pugilator. The ovarian stages corresponding to each moult stage was also studied. Crab was cut opened and the ovaries were removed for observation. The ovary of Emerita asiatica was classified into 3 stages based on the colour and nature of the oocytes in the ovary. Ovarian stages of E.asiatica were classified based on the criteria as adopted by Kerr,(1969), Wolin et al,.(1973).The colour of the ovaries was taken as one of the criteria to determine the ovarian development stage. Overies were carefully removed for biochemical analysis.

RESULT

Characterisation of Molting Stages:

In *E.asiatica* the first sign of molting is the retraction of epidermis is evident even before the hatching of the embryos. The premolt stage is the preparatory stage of molting and during the stage, there is a formation of a new cuticle. The premolt stage advanced further at a time when the pleopod embryos hatch. No female at the time of embryo hatching is in the inter molt stages. During this stage the carapace becomes progressively hard and calcified. After this, the animal starts absorbing water which is distributed within the body. Carapace is lifted by the hydrostatic pressure,ecdysis occurs. Fresh moult-Animal does not feed as evidenced by the empty nature of the hindgut. The cuticle is thin showing bloated appearance. At postmoult stage pigments are associated in the centre. Carbohydrate content increases significantly from freshmoult to postmoult significantly. The amount of Carbohydrate present in the ovary at intermoult is2.93ug/mg. However at Premoult1and premoult

II the value of carbohydrate increases to 3.187ug/mg. The changes in carbohydrate content at different moult stages are significant

Characterisation of Ovarien Stages:

The immature ovary is white in colour and no traces of yolk were observed.Both the ovarian index and total ovarian proteins gradually increase from the inter molt stage maintaining the same level up to spawning. In continously reproducing females ,the protein level increases up to premolt stage with a drastic decline just before ecdysis. The immature ovary is white in color with no traces of yolkwere observed .In next stage, the ovary acquires yellow colour due to the accumulation of yolk granules and gradually becomes light orange and to dark orange(ripe ovary) by further accumulation of yolk.

Changes in the Carbohydrate content of ovary in different Moult Stages of Emerita asiatica

	Fresh Moult	Post Moult	Inter Moult	Pre Moult I	Pre Moult II
	2.73	3.16	2.19	3.05	3.51
	2.83	3.24	2.97	3.52	3.87
	2.82	3.19	2.85	3.12	3.80

	2.89	3.10	3.05	3.12	3.71
	2.74	3.22	2.87	3.13	3.78
MEAN	2.802	3.182	2.926	3.188	3.734
SD	0.679	0.055	0.083	0.188	0.138

Changes in the Carbohydrate content of ovary in different Moult Stages of Emerita Asiatica

ANALYSIS OF VARIANCE (ANOVA)

Source of variation	DF	SS	Mean Sum Of Squares	F=Between Stages/Error Ms
BETWEEN STAGES SS	4	98.83	24.7075	739.1922
WITHIN STAGES SS	20	0.6685	0.033425	
TOTAL	24	99.4985		

Calculated F= 739.1922

Table F at p 0.01= .42

The changes in the carbohydrate content of ovary in various moulting stages are highly significant (p 0.01).

Changes in the Protein content of ovary in different Moult Stages of Emerita asiatica

	Fresh Molt (A)	Post Molt (B)	Inter Moult	Pre Molt I - D_0-D_1	Pre Moult II - D_2-D_{3-4}
	78.03	90.78	100.72	95.00	87.93
	79.33	91.75	105.49	94.95	88.72
	78.57	90.13	105.93	95.22	87.12
	78.61	90.28	108.12	95.35	87.62
	78.47	90.81	105.14	95.82	87.69
Mean	78.602	90.746	106.68	95.268	87.816
D	0.468	0.627	1.627	0.349	0.565

Changes in the protein content of ovary in different moult stages of Emerita Asiatic

ANALYSIS OF VARIENCE (ANOVA)

Source of Variation	DF	SS	Mean Sum of Squares	F=Between Stages/Error MS
Between Stages SS	4	2035.05	508.76	15.37
Within stages SS	20	662.00	33.1	
Total	24	2697.05	--	

Calculated F =15.37

Table F at P 0.01 =4.43

The changes in the protein content of the ovary in various molten stages are highly significant (p 0.01)

DISCUSSION

Moulting event and reproductive event alternate through the life span of the crab(subramoniam1977).Ovarian growth and vitellogenesis occurs mainly during the intermoult period when the animal is carrying their eggs in their pleopods. Soon after the release of the larvae from the pleopod, this anomuran crab enters into quick moulting period. In the freshmoult condition the animal mate (deposition of spermatophore on the sternum) followed by spawning. In other words when moulting occurs the female in variably contains ripe oocyte in the ovary ready for spawning. The changes in the total protein and carbohydrate content reflects on its role in vitellogenesis as well as new cuticle synthesis .whereas the protein rise during the intermolt stage is coincident to the active vitellogenic phase ,the second ramp in the increase of protein during premolt stage may related to new cuticle synthesis (Gunamalai and Subramonium,2002). Although the environmental factors controlling the reproductive cycle of marine crustacean have been delineated (Giese and Pearce,1974),specific interrelationship between molting and female reproductive process is not adequately understood. The adult crab E.asiatica undergoes continuous molting and reproduction throughout the year (Subramonium,1977, 1979; Gunamalai, 2002).In some species, molting cycle synchronised by biological factors such as pheromones (Howe, 1981),the interaction of growth and reproductive hormone system (Scudamore,1948;Conan, 1984),synchronous hat ching(Dagg,1976) are well defined.

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ANTIMICROBIAL ACTIVITY STUDY OF *MARTYNIA ANNUA*

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ABSTRACT:

Martynia annua is native of Mexico and also found throughout India, in waster places, rubbish heaps and road sides. The plant is commonly known as Devil's claw (English), Bichu (Hindi), kakanasika(Sanskrit) and Vichchida (Gujarati).In the present investigation, the leaf and seed of *Martynia annua* were dried and powdered. The extract was subjected to antimicrobial activity with six different species such as *Escherichia coli, Protease mirabilis, Staphylococcus aureus, Salmonella typhi, Klebsiella pneumonia* and *Pseudomonas aeruginosa.* About 30µL, 60µL, 90µL and 12oµL of leaf and seed extract samples were taken and subjected to antimicrobial study with M.H medium. The zone of inhibition was in the increasing order in terms of higher concentration. From the results obtained *Escherichia coli* showed the maximum inhibition than other organisms in the case of leaf and *Protease mirabilis* registered the maximum inhibition in the case of seed.

KEYWORDS

Antimicrobial, zone of inhibition, extract, sensitive, resistance.

INTRODUCTION

The phytochemicals of the plants are found to be the traditional healers for many infectious conditions [1] and metabolites (secondary) are confirmed to have high potency against many infectious disease [2,3.] Several parts of the plants are used as a traditional folk medicine in many countries and proved to have many evidences as medicines or in composition of medicines [4]. The metabolites have proved to be highly inhibitory against many pathogens [5], infections [6] and many disease [7]. In India, different parts of several medicinal plants are used for their therapeutic value from Ancient times to cure many disease [8] and they have proved to be the blueprints for the modern medicines [9]. The bioactive compounds may inhibit the growth or kill the pathogens or cause harm to the host [10,11] or sometimes they become resistant to some drugs [12]. *Berberis tinctoria Lesch* exhibited significant against six bacteria and the maximum activity was against *Pseudomonas aeruginosa* and *Escherchia coli* [13]. Except the root extract *Berberis tinctoria* the other parts of the extracts were inactive against the tested pathogens [14].

Martynia annua is native of Mexico and also found throughout India, in waster places, rubbish heaps and road sides. The plant is commonly known as Devil's claw (English), Bichu (Hindi), kakanasika(Sanskrit) and Vichchida (Gujarati). *Martynia annua* belongs to the family Martyniaceae and it is a small herbaceous, erect, branched, glandular hairy annual herb growing upto 0.9-1.2m in hight. Leaves are large, simple, opposite, green in color, broadly ovate to triangular-ovate, glandular hairy, 9–22 × 9–20 cm, cordate at base with sinuolate-dentate margin and acute apex, sticky as often covered with glutinous dew-like substance (Nagda et al 2009). Glandular hairs exude a slimy sap which gives the plant a clammy feel. Stems are green, robust, branched and covered with glandular hairs. Flowers are drooping, large, pale mauve or lavender in short spikes at the end of branches. They are tubular shaped 4-6 cm long, pink and dark purple blotched with yellow inside, foxglove shaped, ill-smelling and terminate in 5 spreading lobes with a prominent spot between each lobe. Fruits are oblong, green and fleshy when young, becoming black and woody when mature, 3-4 cm long, 1-1.5 cm wide tapering into a long beak (claw), which splits into two sharp re-curved hooks when dry. Claws are shorter than the body of the fruit. Seeds are flat, brown to black, elongated, two seeds to each pod, usually remaining inside the pod (Manandhar and Manandhar 2002). Racemes are long, erect and terminal. Corolla are glandular hairy with very oblique mouth lobes (Kirtikar and Basu 1987). In Ayurveda, the plant is known as kakanasika, which is being used in Indian traditional medicines for epilepsy, inflammation and tuberculosis (Babu et al., 2010). The leaves and fruits are biologically active part of this plant(Chopra et al., 1996)(Satyavati et al., 1987). The fruits of *Martynia annua* used as local sedative and also used as antidote to scorpion stings to venomous bites and stings (Watt 1972). It is commonly known as Bichchhu, used in epilepsy and applied locally to tuberculosis glands of camel's neck. The juice of leaves is used as a gargle for sore throat, fruits used for inflammation, leaf paste has beneficial effect when applied to the bites of venomous insects and wounds of domestic animal (Lodhi and singhai 2011). There in the present investigation, an attempt was made to extract the phytochemicals from the leaves and seed of *Martynia annua* and was subjected to antimicrobial studies with six different organisms.

MATERIALS AND METHODS:

MICROBIAL CULTURE:

- *Escherichia coli*
- *Klebsiella pneumonia*
- *Protease mirabilis*
- *Pseudomonas aeroginosa*
- *Salmonella typhi*
- *Staphylococcus aureus*

A pure culture of the above target organisms were obtained from MTCC, IMTECH, Chandigarh and were cultured in nutrient broth for antibacterial study in the present study. Antibacterial activity study of all the organisms were conducted with Muller and Hinter Agar (Hi-Media Pvt. Ltd. Mumbai) by Kirby-Bauer Disk Diffusion method. The test organisms were swabbed onto the duplicate petri plates, and five wells were made with the help of sterile cork borer. Control and the two target bioactive compounds (Flavonoids and Salicyclic acids) extracted from *Tagetes erecta Linn.* of 150µg and 250µg were pipetted out aspectically and incubated for 24 hours at optimum temperature. The zone of inhibition (in mm diameter) were read after 24 hours and considered as the antimicrobial activity of the test organisms.

RESULTS AND DISCUSSION:

In the presnt study, the leaf and seed of *Martynia annua* were subjected for extraction and then antimicrobial study was conducted with *Escherichia coli***,** *Klebsiella pneumonia***,** *Protease mirabilis***,** *Pseudomonas aeroginosa***,** *Salmonella typhi* and *Staphylococcus aureus.* The various concentrations in both the extracts were 30µL, 60µL, 90µL and 120 µL and the procedure followed was as per standard methods with M.H medium. The results revealed an increase of zone of inhibition with the increase in the concentration of extracts and the results are represented in the Table 1.

Table 1

Antimicrobial activity of *Martynia annua*

S.NO	ORGANISMS	ZONE OF INHIBITION							
		LEAF				SEED			
		30µL	60µL	90µL	120µL	30µL	60µL	90µL	120µL
1	*Escherichia coli*	1.0	1.2	1.4	1.5	0.9	1.0	1.1	1.2
2	*Protease mirabilis*	0.9	1.0	1.1	1.2	0.9	1.0	1.3	1.3
3	*Staphylococcus aureus*	0.9	1.0	1.1	1.2	0.9	1.0	1.1	1.2
4	*Salmonella typhi*	0.9	1.0	1.1	1.0	0.9	1.0	1.1	1.2
5	*Klebsiella pneumonia*	0.9	1.0	1.0	1.1	0.9	1.0	1.1	1.2
6	*Pseudomonas aeruginosa*	0.9	1.0	1.1	1.2	0.9	1.0	1.1	1.2

About six different organisms were subjected antimicrobial activity study with the extract of leaf and seed samples of *Martynia annua.* During the study, there was an increase in the zone of inhibition with the increase in the concentration of extract o. The maximum size (1.5 mm) of zone was observed at the petriplate containing *E*scherichia coli, followed by (1.2mm) with two organisms *Protease mirabilis* and *Staphylococcus aureus* at 120µL concentration. *Salmonella typhi* and *Pseudomonas aeruginosa* registered (1.1 mm) of zone of inhibition and the least inhibition was recorded with *Klebsiella pneumonia* (1 mm), respectively. A maximum zone f 28 mm was registered with *Aduthoda vasicanees* by disc diffusion method in *Staphylococcus aureus* [15] and the minimum 15 mm was observed in *Vibrio cholera*.

During the period of study, the seed extract showed relatively high zone of inhibition than the leaf extract were all the six organisms showed a maximum zone of 1.2 mm except *Protease mirabilis* which registered 1.3 mm at 120µL concentration. It was also surprising that all the lower concentration except 90µL concentration of *Protease mirabilis* recorded 0.9 mm (30µL), 1 mm (60µL) and 1.1 mm(90µL), respectively throughout the study. The above results proved that the biologically active compounds isolated from seed sample are more effective than the leaf sample of *Martynia annua* and it is also very much proved evidence [16].

CONCLUSION

Extract of leaf and seed of *Martynia annua,* has proved to contain high bioactive compounds which showed effective zone of inhibition in all the concentration of the extracts. From the above results, the seed extract showed the maximum effective zones when compared to leaf extract. From the above results it was proved that the phytochemicals of leaf and seed have of high antimicrobial activity and it may prove to be against pathogens on further clinical studies.

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ASSOCIATION BETWEEN LIPID PROFILE AND LIVER FUNCTION TEST

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ABSTRACT :

Lipids are one of the necessary components which control cellular function and homeostasis. Liver plays an essential role in lipid metabolism in several stages and lipid synthesis and transportation. Therefore it is reasonable to expect an abnormal lipid profile in those with severe liver dysfunctions. There is prominent decline in Total cholesterol and HDL, LDL level in patients with severe hepatitis because of reduction of lipoprotein biosynthesis. For reduced liver biosynthesis capacity low levels and LDL, HDL and Total cholesterol is usually observed. Due to the high prevalence of liver disease in our country , The present study aimed to determine lipid profile in patients with liver disease. The study includes 50 patients with Liver disease in comparison with normal lipidemic patients .

KEY WORDS:

Chronic Liver disease, Lipid profile, Liver Function Test, Lecithin–cholesterol acyl transferase

INTRODUCTION:

Chronic liver disease (CLD) affects people in their most productive years of life and has a significant impact on the economy as a result of premature death, illness, and disability[1]. Derangement of serum lipid profile is a common observation in CLD. Very little was known earlier about the alterations of lipids and lipoproteins in patients with CLD.

The liver plays an important role in the synthesis, metabolism and degradation of these lipids and lipoproteins. Hence in CLD the concentrations of these lipids and lipoproteins are altered. There are very few studies on dyslipidemia in CLD in India, but this subject has been dealt in detail worldwide. Although there are vast array of biochemical tests available for diagnosing and assessing severity of liver cell damage, desired sensitivity and specificity are lacking. The routine liver function tests, i.e. serum bilirubin; SGPT used in the assessment of liver function may give abnormal results in various kinds of liver disorders.

Furthermore these tests reflect the extent of hepatic cell damage, rather than hepatic function assessment which is more important to evaluate the patient's condition and prognosis.

MATERIALS AND METHODS:

As the sole aim of this study was to find out the association between lipid profile and Liver Function Test (LFT), we made use of laboratory results available for these patients. All the analytes included in this study were measured using state of art fully automaticanlayser and IFCC reference based test kits. Inclusion or exclusion criteria were not followed as this study was to find out an association between lipid profile and LFT.

RESULTS:

Table 1

Relationship between lipid profile and LFT

Analyte	Liver Damage			Normal LFT		
	N	Mean	± SD	N	Mean	± SD
T.Bil	32	8.2	15.4	35	0.6	0.5
D-Bil	32	5.3	11.2	35	0.2	0.2
Indirect Bil	32	3.0	5.0	35	0.9	6.0
SGOT	32	316.4	1056.8	35	26.7	50.8
SGPT	32	263.9	575.8	35	28.2	38.6
ALP	32	268.6	459.8	35	86.5	60.3
GGT	32	184.1	389.4	35	45.1	156.6
Total Protein	32	6.7	1.6	35	7.1	1.5
Albumin	32	3.5	1.6	35	4.3	1.1
Globulin	32	3.1	1.8	35	2.9	1.1
A/G	32	1.2	0.8	35	1.5	0.7
T.cho	32	112.7	73.6	35	176.1	126.1
TGL	32	182.5	122.4	35	139.5	124.6
HDL	32	19.4	25.2	35	39.9	18.7
LDL	32	74.9	68.0	35	115.5	111.3
VLDL	32	19.8	43.8	35	20.8	19.3
CHO/HDL	32	8.1	11.2	35	4.6	3.3
LDL/HDL	32	4.5	4.2	35	3.0	2.9

Table 2

Lipid profile in CLD and Control group

Analyte	CLD			Control		
	N	Mean	± SD	N	Mean	± SD
T.cho	32	112.7	73.6	35	176.1	126.1
TGL	32	182.5	122.4	35	139.5	124.6
HDL	32	19.4	25.2	35	39.9	18.7
LDL	32	74.9	68.0	35	115.5	111.3
VLDL	32	19.8	43.8	35	20.8	19.3
CHO/HDL	32	8.1	11.2	35	4.6	3.3
LDL/HDL	32	4.5	4.2	35	3.0	2.9

Lipid profiles of the cases and controls were computed and analysed. Data was tabulated in Microsoft excel. The results of this study showed that all the five studied variables (total cholesterol, LDL, VLDL, HDL & TGL) were significantly low in the study population than in the control group. The 3 statistical parameters for all the 50 patients. Very high significant associations were found between HDL vs ALB, HDL vs ALP, LDL vs TP, ALB.

DISCUSSION:

Derangement of serum lipid profile is a common observation in CLD. To the best of our knowledge, there are very few studies on dyslipidemia in CLD in India, but this subject has been dealt in detail worldwide. This study was conducted to document any derangement in lipid profile in CLD patients and whether this derangement has any correlation to severity of liver damage. The results of this study showed that all the four studied variables (total cholesterol, LDL,VLDL, HDL) were significantly low in the study population than in the control group with the exception of serum triglyceride levels. Triglyceride values also showed a decline in CLD patients but it was not statistically significant. The value of serum total cholesterol was significantly lower in patients with CLD when compared to controls in our study. This observation supports the earlier reports. The probable explanation for the reduced serum total cholesterol due to the decline in synthetic function and altered metabolism. This was confirmed in the study conducted by Phillips et al in 1960[2,3,4].

The level of serum HDL in our study was significantly decreased in cases of CLD when compared to control are consistent with a large volume of publications on this subject. Subhan et al[5] observed that in patients with chronic liver parenchymal disease without cholestasis, HDL levels decline and become worse

as the disease progresses. Thus HDL estimation in patients with CLD is an important marker of hepatic function. The decrease in HDL in patients with CLD can be attributed to decreased hepatic synthesis of HDL. This could be due to Lecithin cholesterol acyltransferase (LCAT) deficiency. Liver is the only source of this enzyme (LCAT) and serum levels of this enzyme are decreased in liver disorders. The decreased LCAT results in impairment of conversion of nascent HDL to mature HDL resulting in an increase in immature HDL in blood which is more prone for degradation, resulting in decreased levels of HDL as suggested by VerganiG. Trovati[13]. Neil McIntyre[12] in 1978 also observed that HDL was decreased in patients with Liver parenchymal disease and attributed this decrease in HDL to decreased production of enzyme LCAT[4]. The study also has found that the levels of HDL reduction was proportional to the severity of liver damage in CLD. This HDL reduction is also suggested by Jarikre AE et al, Ahenaku *et al*, Mandal *et al*, Varghese *et al*, Subhan *et al,* and many others studies around the world[6,7,8,9]. There was a significant decrease in levels of serum LDL in patients with CLD, when compared to controls in our study. This is in accordance with previous study by Ahenaku *et al*, Varghese *et al*, Breier C *et al* and Mandal *et al*[10]. But studies by McIntyre[12]. in 1978 observed that the LDL concentration was finding is in keeping with our observations that in severe liver disease as the liver function deteriorates, more decline is observed in LDL, HDL, total cholesterol levels and TGL levels. Our study is comparable in results with other studies like Mandal et al,Subhan *et al* [11], Varghese *et al* [12], Chrostek *et al*[13] and others where they showed a progressive decline in the lipid levels with progression of liver disease.

CONCLUSION:

Hence, dyslipidemia exists in patients with liver CLD and screening for the same is important for intervention with appropriate therapy to prevent cardiovascular events. However, further studies are needed to assess the predictive value of dyslipidemia as a tool to forecast the progression of CLD. Once LFT are altered, it is suggested that additional tests like lipid profile are done to assess the cardiac function.

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EFFECTS OF *ASPARAGUS OFFICINALIS*. L ON INFLAMMATION

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ABSTRACT

Inflammation is the most tremendous disease in worldwide. The medicine for this disease is distributed in various medicinal. But chemical or allopathic medicines are leads some severe side effects in a person. So nowadays, world peoples are turned to alternative for it. One of the method is plant or herbal treatment method. So, this study was designed for investigation of anti-inflammatory effect of Asparagus rhizome on carrageenan induced paw edema in mice. The parameters (paw volume, LPO, GSH, protein, albumin, WBC, Calcium and Magnesium) were analysed. Altered level of this parameters were near normalized by the Asparagus rhizome, indicates its anti-inflammatory or antiedematous activity.

KEY WORDS

Carageenan; Inflammation; *Asparagus officinalis. L; anti-inflamatory activity*

INTRODUCTION

Carrageenan is a high-molecular-weight sulphated polysaccharide that is used in pharmacology to induce local inflammation (paw oedema and pleurisy). It is a pro-inflammatory polysaccharide useful to assess the contribution of mediators involved in vascular changes associated with acute inflammation. Inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. In carrageenan-induced inflammation, the initial phase of inflammation (oedema, 0-1 hr) has been attributed to the release of histamine, 5-hydroxytryptamine and brady-kinin followed by a late phase (1-6 hr) mainly sustained by prostaglandin and proinflammatory cytokine release. Carrageenan local inflammation is linked to neutrophil infiltration and the production of neutrophil-derived free radicals (reactive oxygen species (ROS)], such as hydrogen peroxide, super-oxide and hydroxyl radicals, as well as to the release of other neutrophil-derived mediatory (Population council, 2007) Therefore the inflammation is linked to oxidative stress.

Now a days world population moves towards herbal remedies for treatment of several ailments. The number of plants have been screened for their anti- inflammatory and anti- arthritic activity, but only few of

them reached up to the clinical level. This problem is mainly due to purely academic oriented research. Researchers have to lay emphasis on the phyto constituents obtained form that plant for the specific treatment of such disease and not only to increase the number of plants having anti-inflammatory activity but have to work towards tapping their therapeutic utility.

From very early times plants have been used to cure diseases, and India has a rich heritage of medicinal plants and a fairly comprehensive system of Pharmacopeia. The possibility of naturally occurring compounds showing pronounced antiinflammatory activity in many of these medicinal plants offers a great chance for their economic introduction and utilization in chemotherapy. A large number of Indian plants has been screened for biological activity by Central Drug Research Institute, So, this study was designed for evaluation of anti-inflammatory activity of the plant rhizome *Asparagus officinalis* L.

Asparagus officinalis L belongs to the family *Asparagaceae* family. It is a erect , much branched, dioecious perennial tuberous root used for many medicine preparation. It is used for diuretic, demulcent, tonic, cardiac, sedative, aphrodisiac, fixative. A tincture of the whole plant is used for urinary infections and rheumatism. Roots are more diuretic than shoots. They are recommended in dropsy, enlargement of heart etc. infusion is used against jaundice and congestive torpor of liver. Root bark taken with milk for vitality and strength. Aspargin stimulates kidneys and imparts a strong smell to urine. A decoction of the fresh or dry fruits is used as contraceptive in Europe. Drug is a mild aperient, tonic, aphrodisiac and sedative. It is given in flatulence, calculous affections, dropsy, rheumatism and chronic gout (Joshi,2008). Inspite of its medicinal property, it was selected for screening of anti-inflammatory activity.

MATERIALS AND METHODS

Reagents and Chemicals:

Carrageenan, TBA, DTB like all chemicals were of analytical grade and chemicals required for sensitive biochemical assay were obtained from sigma chemical co., USA. Double distilled water was used in all biochemical assays.

Animal house:

Male albino rats were housed in polypropylene cages and maintained in controlled temperature with standard rat chow. Food and water were provided *ad -libitum.*

Experimental design:

In the study, the rats were divided into three groups of four animals in each group and the body weight of animals were recorded.

Group – I –Normal control (0.5ml of normal saline / animal / day)

Group – II –Rats received carrageenan (1ml / animal) – 0.1 %

Group – III- Rats received carrageenan (1ml / animal) + 100 mg *Asparagus rhizome* for five days.

Collection of samples:

After the completion of experimental regimen, the rats were fasted overnight and blood samples

were collected by cervical decapitation with mild ether anesthesia and serum were collected. And used for various biochemical assays.

METHODS

The level of lipidperoxidation (TBARS)was measured by the method of Nichans and Samuelsson(1968). Glutathione was Determined by Ellman (1959). The total protein in serum was determined by the method of Lowry *etal*.,(1951), and the albumin was determined byBCG method – Rodkey (1965). Calcium and magnesium were diagnosed by OCPC method –Gitelman (1967) and Calmagite method –Gindler *et al* .,(1971). WBC count was done byWintrobe *et al* ., method (1961).

RESULTS

Table 1

Paw edema Volume and Percentage inhibition

Groups	1st hr	2nd hr	3rd hr	5th hr
Control	28.29	34.08	35.06	33.25

Asparagus officinalis L treated group.	12.48 ±7.23	10.2±8.47	9.6±8.98	8.1±8.06

Figure 1

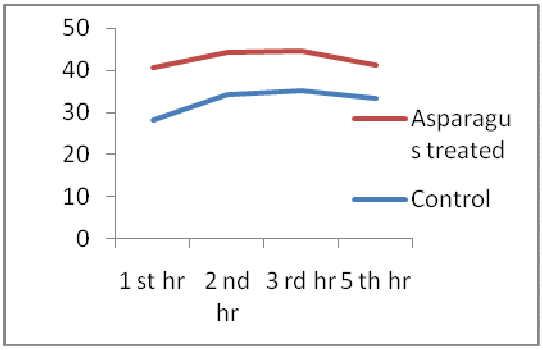
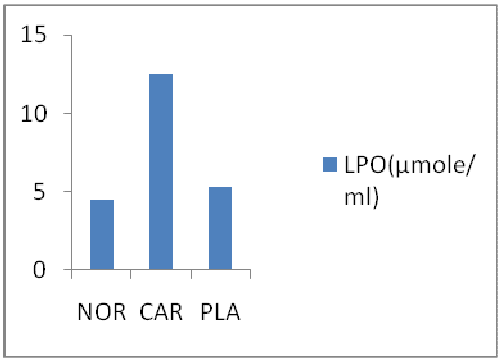


Table 2

Effect of the rhizome of *Asparagus officinalis* L on LPO level in mice serum.

Parameter	Normal	Carrageenan Treated	Rhizome Asparagus treated
LPO (µmole/ml)	4.5±0.51	12.5±0.82*	5.28±0.38#$_2$
GSH (µg/dl)	28.2±0.81	12.2±0.52	24.1±0.52



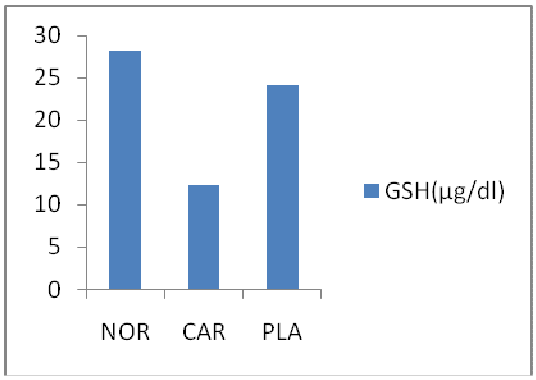


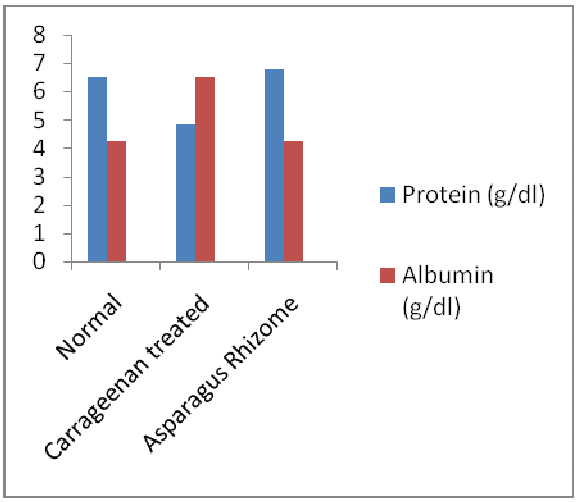
Table – 3

Effect of the rhizome *Asparagus officinalis L* on protein and albumin level in mice serum

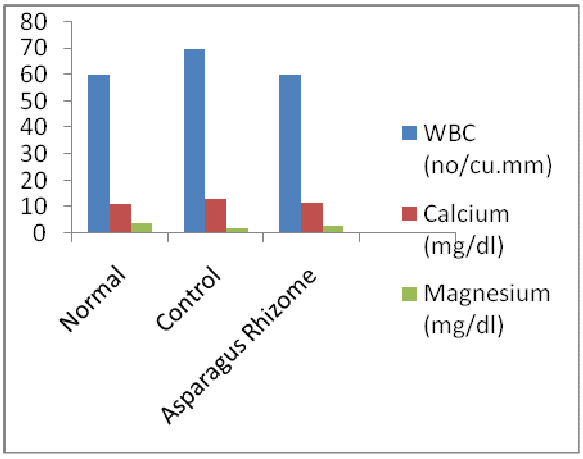
Parameter	Normal	Carrageenan treated	Rhizome *Asparagus* treated
Protein(g/dl)	6.5±0.15	4.85±0.48*	6.8±0.20#$_2$
Albumin(g/dl)	4.25±0.65	6.85±0.75	4.25±0.82

Table – 4

Effect of the rhizomes, *Asparagus officinalis* L *Rottl* on WBC and Calcium and Magnesium level in mice Serum.



Parameter	Normal	Carrageenan treated	Rhizome *Asparagus* treated
WBC(no/cu.mm)	60±2.0	70±5.0*	60±2.0#$_2$
Calcium(mg/dl)	11.2±0.20	13.2±0.80	11.5±0.50
Magnesium (mg/dl)	3.86±0.15	2.1±0.16	3.1±0.15



DISCUSSION

Biological effect of *Asparagus officinalis* on inflammation **Table – I** shows the level of paw volume and percentage inhibition of edeme volume in normal, control and plant drug treated groups of mice. The paw volume was observed in the 3 groups of mice in the time interval of 1sthr, 2nd hr, 4th hr and 5th hr period. In the control group 3rd hr sample had maximum volume of paw volume than others. After 4th hr or 5th hr sample the volume was decreased than maximum limit. In the plant drug treated group the paw volume was decreased than control group. Paw volume inhibition level in As*paragus* group had higher percentage than control group. From this table, we concluded that the anti edematous activity was observed in Asparagus grop when compared with control grop.

Table – II represent the level of LPO and GSH in normal and experimental animals. The level of LPO was increased in control group than other groups, likewise the GSH level was decreased in control group than other groups.

Lipidperoxidation is free radical mediated process. It induces of alteration in structure and function of cellular membrane (Kale, 1995). Reactive oxygen species generated endogenously or exogenously are associated with the pathogenesis of various disease such as atherosclerosis, diabetes, cancer, arthritis and aging process (Corner and Grisham, 1996). Inflammation is a complex process and ROS play an important role in the pathogensis of inflammatory diseases (Guyton *et al.,* 1997, Halliwell and Gutteridge, 1999). And it has been implicated in the pathogenesis of various diseases including arthritis. It is well established that bioenzymes are very much susceptible to LPO, which is considered to be the starting point of many toxic as well as degenerative process.Peroxidation brings about change in structure fluidity and permeability of membranes (Nakazawa and Nagat suka, 1980; Srivastava et al., 1998) inactivates a number of membrane bound enzymes and protein receptors. (Yukawa & Nakazawa, 1980, Yukawa & Naga suka, 1983) induces swelling and alteration of respiratory function (Aono shiraishi, *et al.,* 1981) causes of SH group from the membrane bound protein (Leyko & Bartosz, 1986).

Injection of carageenan into the mice elicited an acute inflammatory responses characterized by accumulation of fluid containing a large number of neutrophils, subsequent lipid peroxidation and increased production of nitrite / nitrate (NOx), PGE_2 tumernecrosis factor – α and enhanced formation of NO by iNOs

may contribute to the inflammatory process. (Tracey *et al.,* 1995, Cuzzocrea *et al.,* 1999) Generation of free radicals and nitric oxide by activated macrophages has also been implicated in causing oligo dendrocyte apoptosis. After tissue injury an animal will display spontaneous pain behaviour and releases various inflammatory mediators bradykinin, prostoglandins or cytokines which can activate and sensitive to the peripheral nerve endings (Ohuchi *et al.,* 1976).

In this present study, the level of LPO increment was due to carageenan damage on membrane lipids and also it may causes nitrite / nitrate production. This reactive species may participate on the swelling process in the rat paw. But the plant drug treated groups had minimized the production of LPO than control group. Likewise the level of GSH was decreased in control group than other groups.

Glutathione is a ubiquitous thiol containing tripeptide, which plays a central role in cell biology. It is implicated in the cellular defence against xerobiotics and naturally occurring deleterious compounds such as free radicals and hydrogen peroxide. Glutathione status is a highly sensitive indicator of cell functionality and viability. Glutathione is the non – enzymrtic antioxidant (Anuradha and Selvam, 1993). Reduced glutrthione undagones oxidation reduction through enzymatic control and inactivities the free radicals. Due to the over utilization of GSH, its level was decreased in control group. But the plant drug treated group was slightly near normalized this GSH level. Both LPO and GSH level alteration may be due to the antioxidative effect of plants drug Asparagus.

Table – III shows the level of calcium and magnesium in normal, carageenan and plant drug treated animal groups. The level of serum calcium was increased and magnesium level were decreased in control group than other groups.

The progression of bone destruction appeared in rheumatoid arthritis is due to increased free radical activity. Carageenan induced paw edema was taken as prototype of exudative phase of inflammation. The development of edema is biphasic (Ghosh *et al.,* 1984). The initial phase is attributable to the release of histamine, serotonin and kinin in the first hour after injection of carageenan. A more pronounced second phase is related to the release of prostoglandins like substances in 2 – 3 hours.During this phases bone destruction may appeared, so the level of calcium was increased and the magnesium level was decreased in

control group, utilization of mg_{2+} for complement pathway may leads to declined level of mg_{2+} in control group. Plant drug was reversed this level in carageenan treated group by its anti-inflammatory activity and antiedematous activity.

Table – IV reflects the level of total protein albumin and WBC in experimental animals. The level of total protein was increased and albumin, WBC were decreased in control group than others.

Mast Cell is a well known effector cell in allergic disease, mediators released from mast cells can cause bronchoconstriction, increased vascular permeability etc, and contribute to the pathophysiolgy of allergic disorders (Galli and Wershil, 1996). Therefore due to the activity of mast cells in allergic reaction accelerates the formation of WBC for this site increased for phagocytosis. This type of reaction may appeared in this inflammatory condition. Therefore the level of WBC was increased in control group than normal, but the treatment of these plant drug on redirected to near normal level. It may be due to its anti-inflammatory activity.

CONCLUSION

The above results confirmed that the *Asparagus* rhizome had anti-inflammatory activity. This activity was accelerated through the antioxidant activity, this mechanism was confirmed through the changed level of LPO and GSH level in treatment group when compared to control group. Finally, concluded that the plant had antioxidant component and properties, and further this plant was recommended for phytochemical and pharmacological activity evaluation.

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CYTOTOXIC ACTIVITY OF THE LEAF EXTRACTS OF *TYLOPHORA INDICA*

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ABSTRACT

Tylophora indica leaf extract was prepared from dried, powdered leaves using the solvents hexane, chloroform, ethyl acetate and methanol. The extract was also studied for its MTT cytotoxic assay against MCF-7 cell line. It gives very strong activity exhibited by ethyl acetate extract, diminished activity in methanol and found weak cytotoxic activity for chloroform and hexane gradually. The cell survival was calculated between viability and cytotoxicity percentage. The data obtained through this study suggests that the leaf extract of seleted plant has potent cytotoxic activity and they possesses many useful bioactive compounds thus making *T. indica* a suitable choice for future research.

KEY WORDS:

Tylophora indica, MCF-7, MTT, Cytotoxicity, Cancer.

INTRODUCTION

Archaeological evidence indicates that the use of medicinal plants dates at least to the Paleolithic, approximately 60,000 years ago. Written evidence of herbal remedies dates back over 5,000 years, to the Sumerians, who created lists of plants. A number of ancient cultures wrote on plants and their medical uses. In ancient Egypt, herbs are mentioned in Egyptian medical papyri, depicted in tomb illustrations, or on rare occasions found in medical jars containing trace amounts of herbs (Nunn, John (2002).[1]

Tylophora indica, which is commonly known as Indian Ipecse, belongs to the family *Asclepiadaceae* (Sabitha Rani *et al*., 2012). It is an endangered medicinal plant that is found in the branching climber found in southern and eastern India. In traditional medicine, it finds its use for the treatment of bronchial asthma, bronchitis, rheumatic pain and dermatitis (Faisal *et al.*, 2005).

MATERIALS AND METHODS

The plant *Tylophoraindica* was collected from Edaiyathur and nearby villages of the polar basin, Thirukkalukundram Taluk of Kancheepuram District. More than 10 kgs of the leaf was collected in March 2012.

Extraction and Drying:

The leaves were air-dried at room temperature for 2 weeks, after which they were ground uniformly into coarse powder. By soaking 50 g of the powdered leaves in 1500 ml of four different solvents namely, Hexane, Chloroform, Ethyl Acetate and Methanol, the extracts were prepared.The filtrate was concentrated under reduced pressure at 40^{o}C for 25min using a rotary evaporator (Superfit-ROTAVAP, India) and the filtrate was used for the following.

MTT assay for cell viability:

The MTT assay (Mossman, 1983) is based on the ability of live but not dead cells to reduce a yellow tetrazolium dye to a purple formazan product. Cells were maintained in DMEM medium, supplemented with 10% Fetal Bovine Serum, at 37^{o}C in humidified atmosphere with 5% CO_2.

The cells were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2 X 10^4 cells/well and allowed to attach overnight at 37^{o}C. The medium was then discarded and cells were incubated with different concentrations of the extracts (15, 100, 150µg) for 24 hours. After the incubation, medium was discarded and 100µl fresh medium was added with 10µl of MTT (5mg/ml). After 4 hours, the medium was discarded and 100µl of DMSO was added to dissolve the formazan crystals. Then, the absorbance was read at 570nm in a microtitre plate reader. Cyclophosphamide was used as a positive control.

Cell survival was calculated by the following formula:

$$\text{Viability \%} = (\text{Test OD} / \text{Control OD}) \times 100$$

$$\text{Cytotoxicity \%} = 100 - \text{Viability\%}$$

RESULTS

Hexane, Chloroform, Ethyl Acetate and Methanol extracts utilized for MTT assay to study percentage of cell viability and cytotoxicity. All have its own and significant value. On MCF-7 cell line hexane extract gives the below values.

Table 1

Percentage of cell viability and cytotoxicity of *Hexane extract* against MCF-7 cell line

Test	Sample (µg)			PC	C
	50	100	150		
% of Viability	73.74936	66.81981	59.8392	23.28994	100
% of Cytotoxicity	26.25064	33.18019	40.1608	76.71006	0

PC- Positive control (Cyclophosphamide), C- Control

It shows gradual decrease in viability of cell and increase in cytotoxic effect reference to varies concentration of extract, 50, 100, and 150 µgs.

Table 2

Percentage of cell viability and cytotoxicity of *Chloroform extract* Sample against MCF-7 cell line

Test	Sample (µg)			PC	C
	50	100	150		
% of Viability	70.7121	62.82542	54.42828	23.28994	100
% of Cytotoxicity	29.2879	37.17458	45.57172	76.71006	0

PC- Positive control (Cyclophosphamide), C- Control

Table 3

Percentage of cell viability and cytotoxicity of *Ethyl Acetate extract* Sample against MCF-7 cell line

Test	Sample (µg)			PC	C
	50	100	150		
% of Viability	59.58397	48.50689	36.95763	23.28994	100
% of Cytotoxicity	40.41603	51.49311	63.04237	76.71006	0

PC- Positive control (Cyclophosphamide), C- Control

Table-3 shows cell viability and cytotoxicity for ethyl acetate extract against MCF-7 cell line, with maximum activity than the other extracts. It shows more than 60% cytotoxicity effect.

Table 4

Percentage of cell viability and cytotoxicity of *Methanol extract* Sample against MCF-7 cell line

Test	Sample (µg)			PC	C
	50	100	150		
% of Viability	64.67586	56.41909	48.44308	23.28994	100
% of Cytotoxicity	35.32414	43.58091	51.55692	76.71006	0

PC- Positive control (Cyclophosphamide), C- Control

Table-4 displays the result of Methanol extract on the cell line, shows more cytotoxic effect than table-1 and table-2. It has nearly about 50% of cell viability and cytotoxic activity, but shows less activity than Ethyl Acetate plant extract.

DISCUSSIONS

Cytotoxicity assays are widely used by the pharmaceutical industry to screen for cytotoxicity in compound libraries. Researchers can either look for cytotoxic compounds. Assessing cell membrane integrity is one of the most common ways to measure cell viability and cytotoxic effects. Compounds that have cytotoxic effects often compromise cell membrane integrity(***Riss TL, et al***). Cell viability is measured by determining the number of living and dead cells in a total cell sample. An increase in cell viability is accompanied by cell growth, while a decrease in cell viability can indicate the toxic effects of compounds.

Below 4 figuers shows the varying effect cell viability and cytotoxicity. Hexane extract and chloroform extract shows least cytotoxic and more cell viable nature. Ethyl acetate and methanol extract has more cytotoxic activity, even ethyl acetate shows more effective cytotoxicity than other with 63% of cytotoxicity. The phytochemical present in Ethyl Acetate plant extract has more anti-cancer activity.

Figure 1

Shows effect of cell viability and cytotoxicity of *Hexane extract* in MCF-7 Cancer cells; C- control; PC- Positive control (Cyclophosphamide).

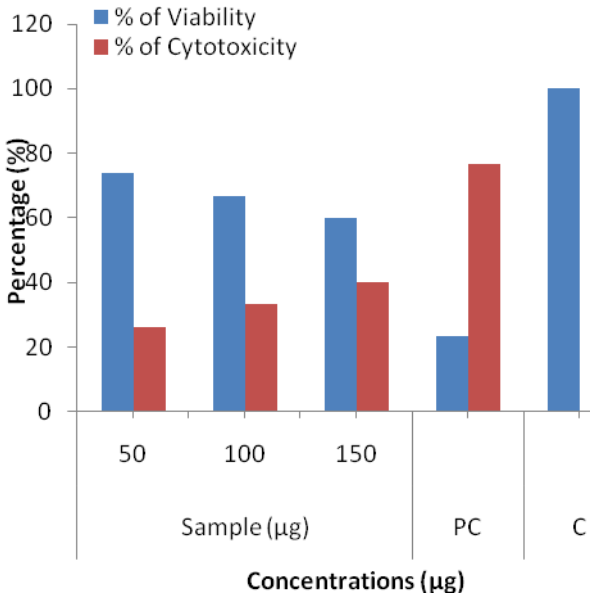


Figure 2

Shows effect of cell viability and cytotoxicity of *Chloroform extract* in MCF-7 Cancer cells; C- control; PC- Positive control (Cyclophosphamide).

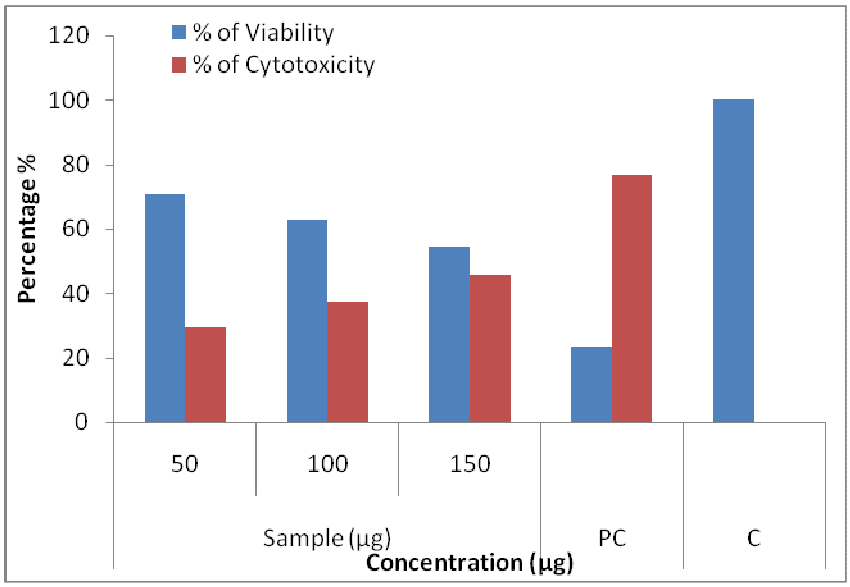


Figure 3

Shows effect of cell viability and cytotoxicity of *Ethyl Acetate extract* in MCF-7 Cancer cells; C-control; PC- Positive control (Cyclophosphamide).

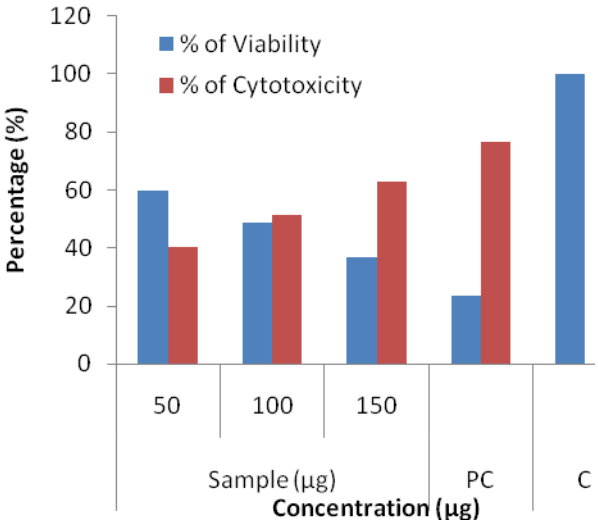
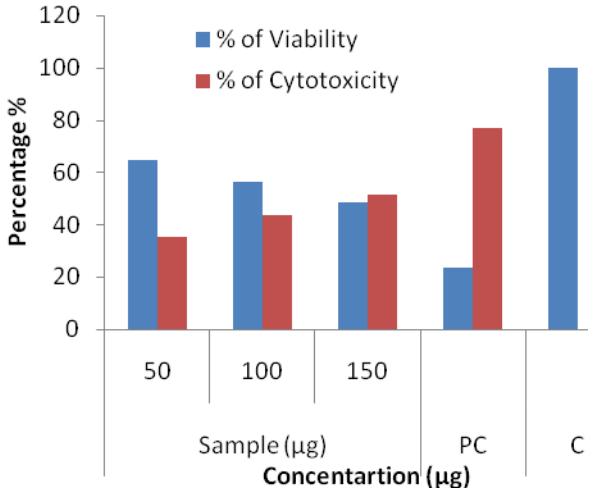


Figure 4

Shows effect of cell viability and cytotoxicity of *Methanol extract* in MCF-7 Cancer cells; C- control; PC- Positive control (Cyclophosphamide).



CONCLUSION

Thylophora indica is the herbal plant commonly used by the traditional healers in tamilnadu. The cytotoxicity and cell viability assay show most effective in Ethyl Acetate than the other extracts. The anti-cancer activity is found this herb, and it will be effective for further research like isolation of pure and bioactive compound identification and commercialization.

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ANTIMICROBIAL ACTIVITY STUDY OF *SENNA ALATA*

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ABSTRACT:

From the historic era, plants have been used as alternate medicine of natural products for human health. The plant metabolites are found to have high antimicrobial properties and several scientific and clinic researches have proved to be folk medicines to treat infections. In the present investigations, the leaf and flower extract of *Senna alata* was subjected to antimicrobial analysis with six different organisms namely *Pseudomonas aeurginosa, Salmonella typhi,Staphylococcus aeureus, Protease mirabilis, Escherchia coli, Klebsiella pneumonia* . From the above result, both the samples (leaf and flower) showed distinct zone of inhibition in the order of increasing concentrations from 30ug to 120ul. The leaf extract showed maximum zone with *Pseudomonas aeruginosa* and the flower extract registered with P*rotease mirabilis* .

KEYWORDS:

Resistance, Sensitivity, Zone of inhibition, Antimicrobial, Muller Hinter media, phytochemicals.

INTRODUCTION:

Nature is a source of various medical plants and proved to be an effective source of traditional and modern medicines[1,2] .From the anicient civilization Egyptians, Chinese,Greek and romans used the extracts of plants for various aliments of human and [animal3,4] .almost all the herbs posses antimicrobial properties and various researches are carried out in girardinia[2] ,tagetes erecta[5] ,sieges beckia orientatus, berberis tinctoria linn[6] , justicia baterica linn,aduthadae vasicances vasicanes[5] and allium kepal.the onion bulbs contains di prophyl disulphide mercaptopropane or prophylmercaptan (flavouring agent) ,allicin cantibiotic,antithrambotic, antihyperteasive),diathyl sulphide (insecticide) dimethyl disulphide (gas odorant) .the garlic bulbs excerted inhibition on the growth of E.coli,pseudomonas pyocyaneus, salmonella typhi, basillus subtilis.

Plant phytochemicals forms the cheapest,safer and alternative sources of antimicrobials[13,14] ,aduthoda vasicanees showed distinct zone of inhibition with asperigellus Niger,aspergillus fumigates and candida albicans[8] .the zone of inhibition was comparated with both the extracts of onion and garlic and

proved to have antibacterial effect on ocular infections[15] .in the present investigation,the leaf and seed extracts were subjected to antimicrobial activity study with six different bacterial species with MH medium.

MATERIALS AND METHODS:

Antimicrobial activity of the plant was carried out with following bacterial strains; *Escherichia coli, Protease mirabilis, Staphylococcus aureus, Salmonella typhi, Klebsiella pneumonia, Pseudomonas aerouginosa.*

RESULTS AND DISCUSSION:

Senna alata plant is found to be wide spread and the extracts of leaf and flower registered high zone of inhibition when it was subjected to antimicrobial activity study. The results of both the smaples were tabulated in the table1. The extracts were pipetted out on sterile condition in their respective wells with negative control in the middle . The leaf extract recorded the maximum inhibitory zone with pseudomonas aeruginasa at 120ul concentration of 2mm diameter zone of inhibition followed by 1.7mm with salmonella typhi and protease mirabilis . The third limit of zone (1.6mm) was registered by protease mirabilis and the last zone of inhibition(1.5mm)was registered by E.coli and staphylcoccus aureus during the period of study. Siegesbeckia orientatis showed moderate activity against *Streptococcus aureus, E. coli, Klebsilla, Psuedomonas*, the root extract of *B. tinctoria* exhibited significant antibacterial activity against *P.* aeruginosa, *E.coli*.[17]

The flower extract of *Senna alata* was found to be relatively sensitive to six bacterial organisms. The maximum zone of inhibition (1.7mm) was observed with *P.mirabilis* followed by *S .aureus* (1.6 mm) and around 1.5 mm was observed with *P. aeruginosa* and *E. coli*, the least zone was recorded with *S. typhi* with 1.4 mm size. Due to the resistance of these phytochemicals against many pathogenic organisms many research and development of new antibiotics are possible from specific plant species [18].

TABLE 1

ANTIMICROBIAL STUDIES OF SENNA ALATA

Organism	**Zone of inhibition** (mm)							
	Leaf				**Flower**			
	30µl	60µl	90µl	120µl	30µl	60µl	90µl	120µl
Protease mirabilis	1.3	1.6	1.8	2.0	0.9	1.2	1.4	1.5
Staphylococcus aureus	1.0	1.2	1.5	1.8	1.0	1.2	1.5	1.6
Pseudomonas aerouginosa	1.0	1.2	1.4	1.8	0.9	1.3	1.5	1.7
Salmonella typhi	0.9	1.2	1.4	1.6	0.9	1.1	1.2	1.7
Klebsiella pneumonia	0.9	1.0	1.3	1.6	0.9	1.2	1.4	1.6
Escherichia coli	0.9	1.0	1.3	1.6	0.9	1.2	1.4	1.5

CONCLUSION:

From the above investigation, the results revealed high significant zone of inhibition in both the samples (leaf and flower extract). The pathogenic organisms tasted brave evidences in developing discases in human and animals. Therefore an further research with extraction of particular compounds will pack a way a well developed evidence to prove that the plant has high therapeutic valve.

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EFFECTS OF THE PLANTS *TRICHOPUS ZEYLANICUS* AND *GOMPHRENA CELOSIOIDES* ON LIPIDPEROXIDATION AND ANTIOXIDATIVE VITAMINS IN DEN/HCB INDUCED LIVER CARCINOGENESIS

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ABSTRACT

Trichopus zeylanicus is a plant with adaptogenic properties. The study was carried out with fourteen groups of rats and was designed to evaluate the beneficial properties of *T. zeylanicus* and *Gomphrena celosioides* consumption on liver marker enzyme regulation and lipidperoxidation in albino rats in DEN/HCB induced stage. For the study, the male albino rats were divided into seven groups normal, chemical treated and chemical along with the crude and ethanol extract of plants treated groups for each phases, initiation and promotion. The Intraperitonial injection of DEN with one day and 83 days treatment of HCB cause carcinogenesis and the other group had plant treatment up to 90 days. Effects of *T. zeylanicus* and *Gomphrena celosioides* consumption on LPO and liver marker enzymes were also evaluated. The plant treatment had remarkable effects on LPO and liver marker enzymes level in the male albino rats. An improvement in vitamins were observed with lower lipidperoxidation and higher vitamins after 90 days of *T. zeylanicus* and *Gomphrena celosioides* crude and ethanol extract treatment. Thus the leaf *T. zeylanicus* and *Gomphrena celosioides* were found to have antioxidant activity.

KEYWORDS

Trichopus zeylanicus Gaertn; *Gomphrena celosioides;*hepatocarcinogenesis; lipidperoxidation; vitamines; male albino rats; antioxidant.

INTRODUCTION

The body posses defence mechanisms against free radical induced oxidative stress which involve physical defence and antioxidant defence. The 'antioxidants' in biology broadly means " a fighter against harmful free radicals". Compounds with antioxidant activity are categorized into three groups namely Excellent, Good and Moderate. Excellent ones are those that perfectly quench the excited state and ground state radicals. Good antioxidants strongly inhibit the peroxide formation but are less effective in quenching excited states. Moderate antioxidants fail to excel in both reactivities (Beautner *et al., 2001*). The

antioxidants from plant products may fall under any of these three categories. The antioxidant activity may also depend on the type and polarity of the solvent.

Trichopus zeylanicus sub-species travancoricus, a perennial herb, belongs to the family *Trichopadaceae* and it is popularly known as '*Arogyapacha*'. *Arogyapacha* is a perennial rhizomatous herb that produce a rosette of 10-15 (Weighting 100-200g) evergreen leaves (with 2 or 3 flush in every year) the leaves were recommended as a sustainable source of materials required for commerial production of the herbal 'Jeevani' medicine.The fruits of this plant is consumed by Kani tribes of Kerala (India) for getting instant stamina, amelioration of old age- related disorders, etc (Pushpangadan *et al*., 1988).

Phytochemical and pharmacological studies on *Arogyapacha* revealed the presence of certain glycolipids and non-steroidal compounds (Polysaccharides)with profound adaptogenic immuno-enhancing antifatigue properties. Three patents on the same were filed by RRL, Jammu. Subsequently investigations, however, yielded similar chemical constituents from leaves of this plant, but in trace around than that present in the seeds. However, harvesting tender fruits of *Arogyapacha* was used for large scale production of Jeevani.

Trichopus zeylanicus Gaertn, belongs to a genus of rare, herbaceous perennial and rhizomatous plants growing along the wet banks of streams and rivulets in tropical forests in India, malaysia and Ceylon (Pushpangadan *et al*., 1988). In India *Trichopus zeylanicus Gaertn* is a wild plant growing in the Agasthyar hilly forests of Kerala, used this plant as a health tonic and rejuvenator. The fresh fruit kernels of *Trichopus zeylanicus Gaertn* are eaten by the kani to obrain instant energy, stamina and vitality. It is claimed that those who ingest the Kernels can live for days without food and with very rigorous physical work. It is further claimed that by taking one or two fruits daily a person can always remain healthy, agile, young and immune to diseases (Pushpangadan *et al*., 1988). Pharmacological work has ever been reported for reports of anti-stress activity of a 70% ethanolic extract of seeds of *Trichopus zeylanicus Gaertn* in rats and mice (Sharma *et al*, 1989) and hepatoprotective activity in rats (Subramoniam *et al*., 1988).

MATERIALS AND METHODS

Reagents and Chemicals :

DEN, HCB, TBA, DNPH, dipyridyl like all chemicals were of analytical grades and chemicals required for sensitive biochemical assay were obtained from"M/S sigma and Aldrich chemical co., U.S.A.," Double distilled water was used in all biochemical assays.

Animals:

Albino wister rats of male at a age of 15-20 weeks containing 120-150g weight were selected for this study.These animals were purchased from Indian Institute of science,Bangalore, India. Male albino rats were housed in polypropylene cages and maintained in controlled temperature with standard rat chow. Food and water were provided *ad -libitum.*

DEN/HCB and Cornoil Dosage in Carcinogenesis Induction:

DEN : 20 mg in saline/Kg for only one i.p injection.

HCB : 0.4 mmole /1ml cornoil from 2^{nd} week to 12^{th} week.

Cornoil: 1 ml per day.

Preparation of Plant material and extracts:

Trichopus zeylanicus and *Gomphrena celosioides* were collected and shade dried for grinding to get crude powder for treatment. These plants were shade dried and extracted with ethanol (70%) by the use of soxhlet extractor. A semisolid extract was obtained after complete elimination of ethanol under reduced pressure. The extract was stored in refrigerator untill use. The extract was dissolved in normal saline just before oral administration.

Both the plants were identified by Dr.Jegadeesan,Professor for Herbal and Environmental Science, Faculty of Sciences, Tamil University, Thanjavur and voucher specimen were kept at the department for further verification.The voucher number for plant-1)*Trichopus zeylanicus Gaertn* is – TUH- 287 and plant-2)*Gomphrena celosoides C.Martius* is-TUH-286.

Experimental design:

The rats were divided into fourteen groups of four animals in each group and the body weight of animals were recorded. Four days fasting and refeeding was continued, before the administration of DEN injection.

Batch I-Initiation phase:

Group – I:Normal control (0.5ml of normal saline / animal / day) up to one week.

Group – II :Rats received corn oil vehicle (1ml / animal / day) up to one week.

Group – III :Rats received only one i.p injection of DEN (20mg in saline/ rat) at 1st day of first week.

Group – IV :Received DEN only + *Trichopus zeylanicus* crude powder (200mg/kg) upto one week.

Group – V:Received DEN only + *Gomphrena celosioides* crude powder (200mg/kg) upto one week.

Group – V1: Received DEN only + *Trichopus zeylanicus* ethanol extract (50mg/kg) upto one week.

Group – V11:Received DEN only + *Gomphrena celosioides* ethanol extract (50mg/kg) upto one week.

Batch II-promotion phase:

Group –1 : Normal control Rats received (0.5ml of normal saline / animal / day) up to 90 th day .

Group –1I:Rats received corn oil vehicle (1ml / animal / day) up to 90 th day.

Group –III:Carcinogenic group : Received DEN + 0.4 mmole HCB in cornoil vehicle from 2nd week to 90th day.

Group – IV :Rats received DEN+HCB similar to that of Group – III along the treatment of *Trichopus zeylanicus* (200mg/kg) crude powder/day upto 90th day.

Group –V :Rats received DEN+HCB similar to that of Group – III along the treatment of *Gomphrena celosioides* (200mg/kg) crude powder/day upto 90th day.

Group – VI :Rats received the DEN + HCB + treatment of *Trichopus zeylanicus* ethanol extract (50mg per kg) per day upto 90th day.

Group –VII :Rats received the DEN + HCB + treatment of *Gomphrena celosioides* ethanol extract (50mg/kg) per day upto 90th day.

Collection of samples:

After the completion of experimental regimen, the rats were fasted overnight and blood samples were collected by cervical decapitation with mild ether anesthesia and serum was collected. whole liver was immediately dissected out and washed in ice cold saline. A known weight (1g) of liver was taken and homogenized with (10%) phosphate buffer (pH. 7.4). The serum, whole blood with EDTA, and liver homogenate were used for various biochemical assays.

Biochemical analysis:

The serum and liver was used for the estimation of LPO,VIT-C,E and A.. The level of lipidperoxidation (TBARS) was measured by the method of Nichans and Samuelsson (1968). The content of Vitamin – A. C.E were measured by Neeld and Pearson (1963), Desai (1984) and Omaya *et al* (1979) respectively.

RESULTS

Effects of *Tz* and *Gc* on Lipid-Peroxidation:

Table-I depicts the level of serum and liver LPO.In chemical control groups LPO was significantly ($P < 0.05$) increased when compared with the values of the normal control rats. When the plants *Trichopus* and *Gomphrena* were supplemented throughout the study, LPO level was significantly decreased.

Effects of *Tz* and *Gc* on antioxidant vitamins:

The Concentration of Serum Vitamin E ,C and liver vitamin A were given in **table –II** and **III**. The level of vitamin E was nonsignificantly and vitamin C was significantly ($P>0.05$) decreased in DEN group and these were significantly decreased in DEN+HCB group of rats serum, when compared with normal group. Liver vitamin-A level was significantly decreased in DEN group and increased in DEN+HCB group. The plants *Tz* and *Gc* administration nearnormalized these value when compared with chemical control indicates its antioxidant effect.

TABLE: 1

PARAMETER	GROUPS	INITIATION		PROMOTION	
		SERUM	LIVER	SERUM	LIVER
LPO	N	8.995±0.76	10.19±7.6	27.97±0.164	35.9±0.11
	O	12.19±0.76*	20.99±10.0*	32.7±0.16*	52.7±0.18*
	C	64.79±0.92*	104.9±7.7*	57.55±0.04*	66.7±.16*
	A	19.79±1.11**	64.39±10.4**	38.3±0.58**	28.8±.37**
	B	25.39±1.80**	59.99±13.8**	39.1±0.84**	43.1±0.18**
	A1	18.61±1.02**	54.21±9.20**	50.4±.446**	39.17±0.15**
	B1	24.21±1.60**	54.01±12.6**	45.5±0.14**	33.52±0.57**

Values are the mean ± SD of 4 animals in each group.

Group II and III were compared with Group I (*P<0.05)

Group IV, V, VI and VII were compared with Group III (**P<0.05)

TABLE: 2

PARAMETER	GROUPS	INITIATION		PROMOTION	
		SERUM(vit-c)	SERUM (vit-E)	SERUM (vit-c)	SERUM (vit-E)

VIT-C and VIT-E	**N**	4.02± 0.15	3.3±0 .076	4.10±0.076	2.584±0.02
	O	5.68±0 .065*	5.8±0.190^{NS}	5.14±0.04*	5.23±0.009*
	C	3.66± 0.016*	2.94±0.164 ^{NS}	1.46±0.04*	1.13±0.08*
	A	5.98± 0.016**	4.84± .080 ^{NS}	8.48±0.11 ^{NS}	2.0±0.05**
	B	4.12± 0.46**	5.24± .083**	4.44±0.03 ^{NS}	1.63±0.004**
	A1	5.52±0.112**	6.25±0.20 ^{NS}	4.44±0.26 ^{NS}	1.585±0.017**
	B1	4.10±0.04**	6.26±12.0**	4.02±0.085^{NS}	1.361±0.0331**

Values are the mean ± SD of 4 animals in each group.

Group II and III were compared with Group I (*P<0.05)

Group IV, V, VI and VII were compared with Group III (**P<0.05)

TABLE:3

PARAMETER	**GROUPS**	**INITIATION**		**PROMOTION**	
		SERUM	**LIVER**	**SERUM**	**LIVER**
	N	2.850±0.04	4.501±0.05	3.215±0.06	4.804±0.07
	O		4.150±0.07*		4.296±0.15*

		2.721±0.06		3.150±0.16	
VIT-A	**C**	1.952±0.02	3.56±0.02*	6.820±0.19	9.5531±0.18*
	A	2.015±0.02	3.81±0.29**	4.250±0.29	6.289±0.39**
	B	2.821±0.15	4.12±0.12**	5.120±0.15	7.382±0.14**
	A1	2.525±0.20	3.80±0.20**	4.520±0.27	6.255±0.35**
	B1	2.950±0.10	4.01±10.0**	5.112±0.15	7.125±0.15**

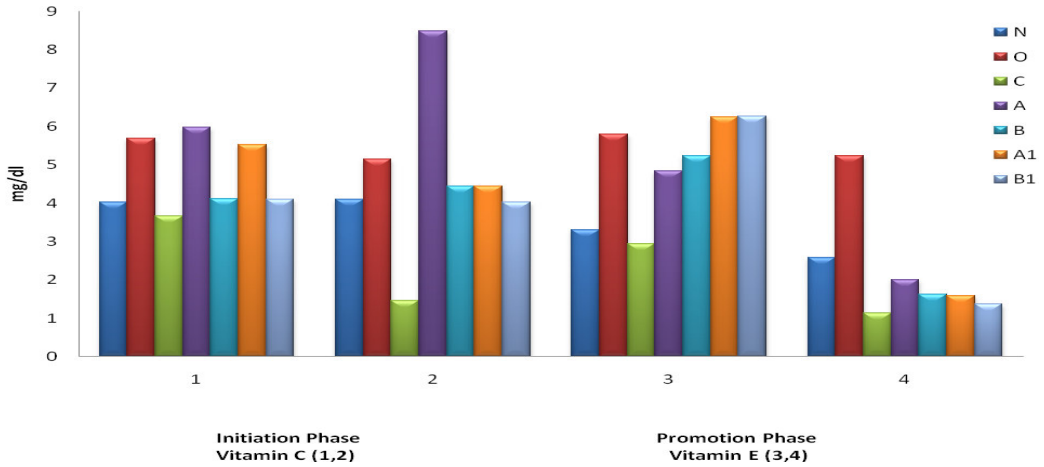
Values are the mean ± SD of 4 animals in each group.

Group II and III were compared with Group I (*P<0.05)

Group IV, V, VI and VII were compared with Group III (**P<0.05)

Figure 1

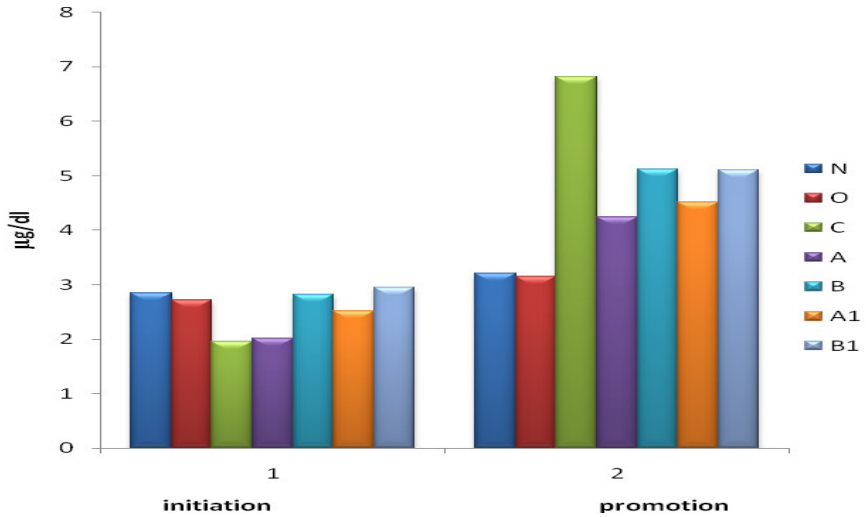
Effects of the Plants *Tz* and *Gc* **on Serum antioxidant Vitamins C**



Values are the mean ± SD of 4 animals in each group. Group II and III were compared with Group I (*P<0.05) Group IV, V, VI and VII were compared with Group III (**P<0.05).

Figure 2:

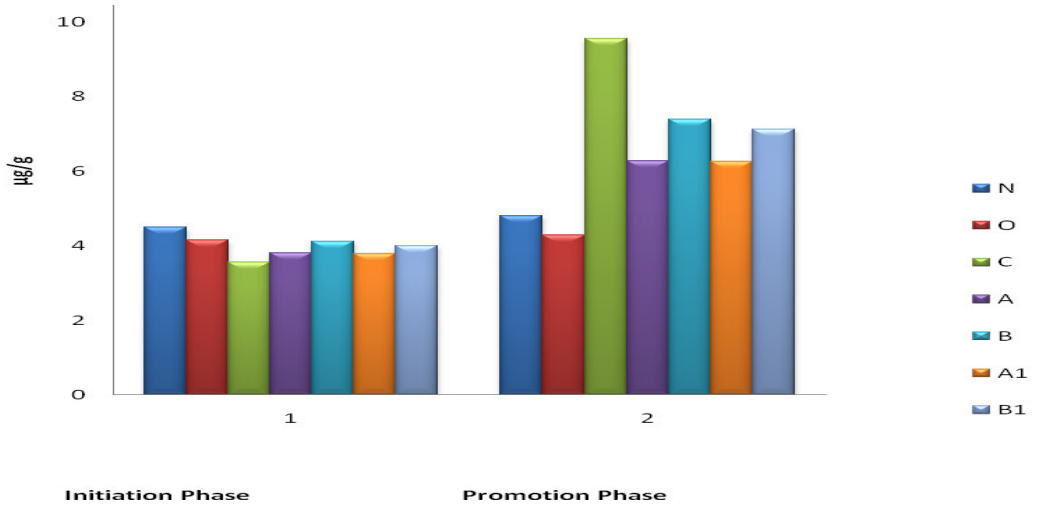
Effects of the Plants *Tz* and *Gc* **on antioxidant Vitamin – A in serum**



Values are the mean ± SD of 4 animals in each group.
Group II and III were compared with Group I (*P<0.05)
Group IV, V, VI and VII were compared with Group III (**P<0.05).

Figure 3

Effects of the Plants *Tz* **and** *Gc* **on Liver antioxidant Vitamin - A**



Values are the mean ± SD of 4 animals in each group.
Group II and III were compared with Group I (*P<0.05)
Group IV, V, VI and VII were compared with Group III (**P<0.05)

DISCUSSION

Vitamin also directly scavenge ROS and upregulate the activities of antioxidant enzymes. Among them vitamin-E has been recognized as one of the most important antioxidants. Vitamin-E inhibits ROS induced generation of lipid peroxyl radicals, thereby protecting cells from peroxidation of PUFA in membrane phospholipids, from oxidative damage of lipoproteins, cellular protein, DNA and from membrane degeneration. consequently, a dietary deficiency of vitamin-E reduces the activities of hepatic catalase, GSH peroxidase, and glutathione reductase (chow et al., 1969) inducing liver lipid peroxidation.

As a reducing agent, vitamin-C reacts with a vitamin-E radical to yield a vitamin-C radical while regenerating vitamin-E. Like a vitamin-E radical, a vitamin-C radical is not a reactive species because its unpaired electron is energetically stable. A vitamin-C radical is converted back to vitamin-C by GSH.Vitamin-E plays a vital role in reducing the incidence of cancer. It is a powerful chain breaking antioxidant, inhibits lipidperoxidation (Halliwell and Gutteridge., 1985). This process is important in maintaining the integrity of all membranes.

An inverse association between serum vitamin-E and the risk of cancer has been reported (Suresh and Vasudevan., 1994). Vitamin-E quenches singlet oxygen and reacts with superoxide radical. Vit-E will

scavenge peroxyl radicals which arise in the lipid or appear as secondary radicals from the hydroxyl radical scavengers. It is a very effective radio protector of micellar fatty acids. Vitamin–E has been reported to specifically modify signal transduction at several steps, including PLC and arachidonic acid metabolism (Balasubramniyam et al., 1994).

Vitamin-C (ascorbic acid) is a water soluble vitamin, that is able to act as a strong reducing agent, which is required for detoxification of various xenobiotics derived organic radicals *in vivo*. Frei *et al* (1989) demonstrated that ascorbic acid is one of the major physiological antioxidants essential for the production of humans against disease and degenerative disorders caused by oxidative stress. In addition, vitamin-C is an effective scavenger of aqueous peroxyl radicals in plasma and normally provide a significant health benefit at a certain concentration range. Ascorbate, can scavenge most ROS, including $O_2^{0-,}$ $OH^{0,}$ RO_2^0 and ONOOH as can GST (Halliwell and Gutteridge., 2006). vitamin-C has also been shown to be an anticarcinogen in rodents treated with benzopyrene and nitrite, which are the major toxic compounds in cigaratte smoke (Frei et al., 1989). Vitamin-C help to maintain a healthy immune system prevents mouth, throat, stomach and intestinal cancers by neutralizing cancer promoting nitrosamines (Kessler *et al.,* 1992).

Vitamin-A and β-carotene are also known to act as potent antioxidants and are also shown to interfere with the peroxidative process. β-carotene plays an important role in reducing the peroxidative process and the incidence of cancer (Peto et al., 1981). Two intriguing properties of β-carotene are the ability to trap certain organic free radicals, and to deactivate excited oxygen molecules, which are generated as a biproduct of many normal metabolic process. The free circulating carotene could have a direct protective effect against carcinogenesis. In experimental animals, vitamin-A deficiency has been shown to lead to premalignant changes in the respiratory, GI and genitourinary tract, in addition, deficiency increases the suscipility of animals to induce cancers of the oral cavity, lung, bladder and colon (Ostrowski et al., 1987). β-carotene act as antioxidants under normal physiological conditions, it can also act as prooxidant at high concentration and more oxidizing conditions.

The study of sundaresan and subramanian (2003), showed that the intoxication of rats by N-nitrosodibutyllamine was accompanied by a significant decrease in the levels of β-carotene, ascorbicacid, vit-E, reduced GSH, SOD and catalase. The above findings suggested that Vitamin-C and Soybean

supresses DBNA induced hepato-urino-carcinogenesis, may be modulating the antioxidant defence status of the animals in response to the antioxidant action of their contents of flavonoids and polyphenolic compounds. The present finding also revealed that, the level of vit-A, C and E were decreased in DEN, DEN+HCB group than normal group. Generally the natural antioxidants strengthen the endogenous antioxidant defence from ROS ravage and restore the optimal balance by neutralizing the reactive species.Similarly the plant extracts *Trichopus* and *Gomphrena* revealed its anti lipidperoxidative effect by incrementing of these vitamins (A, E,and C).

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ANTIBACTERIAL ACTIVITY OF TWO MARINE ALGAL SPECIES

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ABSTRACT:

Marine algae are known to produce a wide variety of bioactive secondary metabolites and several compounds have been derived from them for prospective development of novel drug by the pharmaceutical industries. In this context, algal isolates from **Covelong beach, Chennai, India** were evaluated for antibacterial activity against few bacterial species. Experiment on extracts from red and green algal species revealed its specific antibacterial activity. Agar well diffusion technique was carried out on methanol extracts of two algal species and its zone of inhibition against few of the bacterial species were compared in the study to evaluate the toxicity level of the algal extract concentration against different bacterial species. When challenged with few pathogens. *P. aeruginosa* was found to be the more sensitive bacteria in comparison to four others. The selective toxicity of certain marine algae species may be the specific character of the species. Further, this study will pass some light on the microbial diversity of the marine region.

KEY WORDS:

Marine algae, secondary metabolites, antibacterial activity, well diffusion method

INTRODUCTION

Marine algae diversity of Tamil Nadu is next only to Gujarat cost in India. Algae contain a rich and largely entrapped source of a vast assortment of biologically active substances. This active metabolites, known as biogenic compounds, produced from several species of marine macro and micro algae have some antibacterial, antialgal and antifungal properties. A number of antimicrobial compounds have been identified in microalgae as well as microalgae. Particularly marine algae were reported to produce a wide variety of bioactive secondary metabolites as antimicrobial, antifungal, antihelmintic, and cytotoxic agents and the bioactive substances included alkaloids, polyketides, cyclicpeptide, polysaccharide, phlorotannins, diterpenoids, sterols, quinines, lipids and glycerol's. *Cholorodesmis fastigiata, Dictyota dichotoma* both are species of marine algae in the class *ulvophyceae* under phylum *chlorophyte* and into class *phaeophyceae* under phylum *ochrophyta* respectively.

In the study, we have investigated the antibacterial activity of these two species of algae from the costal area of Covalong beach, Chennai, India. We have ascertained the optimum volume required to be effective in antibacterial activity. Marine macro-algae are considered as the actual producers of some compounds with high bioactivity.[1]

MATERIALS AND METHODS:

COLLECTION OF MARINE ALGAE SAMPLE

Both of *Dictyota dichoma* and *Chlorodesmis factigita* species were collected from the costal and deep sea area of Covalong beach, Chennai, India. This area is related to coromandel cost of Bay of Bengal. The samples were collected during the month of December. Both the algae sample collected live and healthy were identified by expects from algal specialized study. These two identified sample were rinsed with fresh water to remove epiphytes and necrotic parts. Hard portion of the sample were removed. Finally the algae sample were separately socked in distilled water for 2hrs. Later the water part is drained down completely from the sample before allowing them to get free from water contents. Around 12 hrs time was given for each sample at room temperature before extraction process is started. Direct sun rays were avoided to preserve the antibacterial characters.

EXTRACTS PREPARATION:

Both the sample were ground finely after chopping them individually. Sterile pestle and motor was used for grinding the samples. 75% methanol being a good organic solvent, to enhance this solvent extraction process, 48hrs time with excess methanol was allowed at 37ºc. Sterile glass rods were used for even mixing of the extract to improve the extraction process. Finally after 48hrs at 37ºc with intermittent mixing at every 6hrs, filtration was done using Whatman filter paper No:1. Sterility is maintained in all the extraction and filtration process to avoid any bacterial or fungal contaminants.[2]

VIABLE BACTERIAL SUSPENTION:

Pure culture of five different species of bacterial strains were isolated out with care from individual special media (Agar plates). Sterile Muller Hinton agar plates were kept ready at 37ºc to inoculate all the five species. Species *E.coli, P.aeruginosa, S.aureus, S.typhi, K.pneumoniae* were sub cultured individually in nutrient broth to maintain even quantity of bacterial suspension. Brown's opacity tube or MC Farland's opacity tube were used to maintain a standard to quantitatively examine the volume of organism. Very young 24hrs inoculatm of sub cultured bacterial suspension(pure culture) were inoculated in the Muller Hinton agar plates individually.[3] Each organism was inoculated into 12 plates for 5 species, totally (12×50)=60 plates were incubated two minutes after inoculation of the sub cultured species. Pure culture of the specific species is ensured in every part of the experiments.

OPTIMUM CONCENTRATION OF THE EXTRACT:

Both the extracts were diluted suitably to attain an appreciable concentration. Final concentration was maintained exactly at a level equal to 5g dry algae per 50ml solvent, which may be estimated to be around 20% algae suspension after excluding the dry residue's mass. Methanol being the solvent, evaporation need to be controlled by proper covering. After allowing the extract for 48hrs, each sample of extract were filtered again using Whatman filter paper No 1 to separate the filtrate to test the antibacterial activity. Aquatic organisms are a rich source of structurally novel and biologically active compounds.[4] Since the extraction is complete in a concentration equal to 100mg/ml examined by others.

ANTI BACTERIAL ACTIVITY:

Agar well diffusion test technique was carried out on sterile fresh Muller Hinton Agar (MHA) medium with inoculated with five young bacterial suspension of even quantity of using bacterial suspension.[5] All the (25×2) Muller Hinton agar plates were diged 10mm dia wells using suitable sterile cork borer. It was ensured that the cork borer never touch the bottom of the MHA medium or petri plate. For comparing the antibacterial activity of these isolated algae extracts with its therapeutic action, a few broad spectrum antibiotics discs of standard concentration were used for this 5 species Oxicilline-10 mcg/disc, Bacitracin 10 unit/disc, Nalidaxic acid 30mcg/disc were used to examine the inhibition. This antibiogram study is used as a check to estimate the quality of antibacterial activity.[6] All the 50 plates were inoculated with the available evenly quantified pure culture suspensions of (10 plates each species). Necessary care was taken to avoid contaminating the wells with the bacterial suspension. Different concentration volume of each algal extract was added to the respective well on the Muller Hinton agar plates. Special attention was given while making Muller Hinton agar plates and making wells on the agar plates.[7]

a. Agar concentration was maintained at 0.5% higher than regular Muller Hinton agar preparation routinely used for diagnostic bacteriology.
b. The thickness of the medium was maintained at 2-3 mm more than the regular thickness of petri plate poring.
c. 10 mm dia wells were made instead of routinely made 5mm dia wells to encourage better diffusion process.
d. Depth of the wells were cautiously maintained evenly to make sure that the well's bottom does not be made up to surface of agar plates to avoid dispersion of algae suspension through the bottom surface of glass plate. Preferably ½ the thickness of the agar was diged with care.

The concentration varying from 30µl to 120µl volume were placed in the wells apart from a control well with methanol.[8] Apart from this 3 antibiotic disc were also placed over the inoculated bacterial suspension before allowing to diffuse at room temperature for 30 minutes. Each labeled plates was placed at

inoculation temperature for 24hrs with aluminium foil cover to prevent evaporation of methanol due to temperature.[9]

RESULT

The diameter of the zone of inhibition was measured in millimeters. The zone exhibited by the algal extracts of different volumes were compared to the inhibition zone produced by the antibiotics.[10,11] The clearance developed in each bacterium species is recorded and the average inhibition by the 3 antibiotic disc is produced below. The clear zone greater than 10mm dia were considered as positive.[12] Inhibition less than 10mm measurement was interpreted as "TRACE ACTIVITY EXTRACTS" and diameter between 11mm and 25mm was interpreted as "ACTIVE EXTRACTS" sample exhibiting more than 26mm dick inhibition were expressed as.[13,14]

In the table S. No 1 is *Dictyota dichotoma* and S. No 2 is *Chlorodesmis fastigiata* are extracts concentrations in 120µl, 90µl, 60µl, 30µl, control(75% methanol) toxicity.[15]

S. No	Bacterial Species	120µl	90µl	60µl	30µl	Control	Oxicilline	Bacitracin	Nalidaxin
01	*S. aureus*	20	17	19	20	6	17	13	19
	S. typhi	14	15	15	13	8	16	12	18
	P.aeruginosa	29	26	20	21	7	15	16	20
	K.pneumonia	18	19	21	17	9	13	17	13
	E. coli	14	15	13	14	6	16	19	18
	S. aureus	16	13	10	15	5	16	12	17
	S. typhi	11	11	10	13	9	17	9	13

02	*P.aeruginosa*	16	18	17	19	8	15	15	15
	K.pneumonia	15	13	18	12	8	14	14	11
	E. coli	13	12	15	16	6	16	14	22

DISCUSION

Chlorodesmis fastigiata (C. agardh) is a marine species of plantae kingdom and Dictyota dichotoma (Hudson) of chromista kingdom were marine benthic flora of this region. This study on algal suspension in methanol (75%) proved to be a better extraction and methanol was inhibiting the bacterial growth to a minimum level only.[16,17] To enhance the diffusion of antibacterial effect and to improve the techinique the following technical changes were made from the previous experiences of other workers.

a. Diameter of the wells were increased to 10mm diameters instead of 5mm dia preferred by earlier workers.
b. Wells were diged only up to half deep from the surface of the media to prevent the extract touching the petri plate's bottom.
c. Agar agar concentration was 0.5% increased in the Muller Hinton agar medium to facilitate the surface and the well as well as medium to give a firm well and their by hold the suspension.
d. Added 3 antibiotic discs in every plate gave a valid information on the antibacterial activity of each species. Its average inhibiting effect was reliable.
e. Controls for each species helped in understanding the solvent nature and its antibacterial effects on the species taken for examination.[18]

From this study organic solvents like methanol always have higher efficiency in extracting anti-bacterial compounds from the extracts of algae. All the gram positive and gram negative pathogenic bacteria taken for their study are sensitive to the algae extract.

CONCLUSION

Microbial diversity of this marine region near Chennai is safe in the hands of available algae species in this region. Further advanced extraction study and separation and isolation of compounds which all toxic to microbes present in these algae will help the clinicians to treat some bacterial species become resistant to normal antibiotics.[19,20] Above all this study may direct a way for further developments in biopharmaceutical potentiality of marine algal species. Further study on phytochemical is very much needed to understand the vital compounds present in the algae and also to use them as an alternative to synthetic and conventional antibiotic. If study on mixed or combined antibacterial effect of two or three algae species with different solvents enhancements or interaction of antimicrobial compounds against resistant stains of bacteria and micro bacterium species will support medical practionees and patients to a greater level. Experiments on animal modules with suitable extraction techinique will be beneficial to man kind.

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SCREENING AND EVALUATION OF BIOACTIVIE COMPOUNDS FROM *SENNA ALATA* BY GC-MS ANALYSIS

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ABSTRACT

Two third of the World's plant species (3,500 Nos.) are known for its specified therapeutic values and they have normal metabolic activities in human and specific functions within the body. In the present study, *Senna alata* plants were collected, segregated, air dried and powdered. The powdered stem and leaves were subjected to methanolic extraction. The methanolic extraction after filtration, were subjected to GC-MS analysis. The leaf and stem extract registered 9 and 6 phytochemicals, respectively. It was also noted that the active compounds registered in both the samples were different and they are of high medicinal value.

KEYWORDS:

Gas chromatography, Mass spectroscopy, screening, bioactive compound, therapeutics.

INTRODUCTION:

Traditionally, man started using the potent biochemical from plants and utilized for various purposes in industries [1,2]. Indian medicinal systems since from vedas, evidenced the use of secondary metabolites of plants for curing many human and animal aliments [3,4]. The phytochemicals are obtained from various parts of the plants (bark, leaves, leaves, flowers, roots, fruits, seeds etc.)[5] and they are all taxonomically distinct among the plant kingdom [6]. Secondary metabolites are classified on the basis of chemical structures containing nitrogen or not their solubility in various solvents or pathways by which they are synthesized [7]. Alkaloids are of low molecular weight cyclic organic compounds containing nitrogen in a negative oxidation state which has a inducible defense response to animal or insert herbivore and are limiter distribution among living organisms [8,9].

GC-MS analysis is a separation technique in which the plant bioactive compounds are separated in a suitable mobile phase and proved to be highly therapeutics in the field of pharmacy [10.11]. Around 17 phytochemicals were registered in the methanolic leaf extract of C*assia italica* [12]. The phytol diterpene with RT 19.67 is known for its antimicrobial, anticancer, anti-inflammatory and diuretic agent etc. [13] and hexadeconoic acid, 1,2, Benzene dicarboxylic acid and di isoctyl ester were present in C*aesalpinia sappon* ethanol extract [14]. In the present investigation, the methanolic extract of leaves and stem were subjected to GC-MS analysis as per standard procedures.

MATERIALS AND METHODS:

Senna alata diseased free plants were collected segregated into leaf and stem washed thoroughly air dried, powdered and stored in an air tight containers for further investigation. The methanolic extract of leaf and stem were injected with syringe to screen and estimate the total bioactive phytochemicals in GC-MS analyzer as per standard methods.

RESULTS AND DISCUSSIONS:

The methanolic extract of leaf and stem of *Senna alata* registered about nine and six phytochemicals, respectively during the period of study and the GC-MS analysis result obtained is recorded in the Tables 1 and 2. The leaf extract registered around nine phytochemicals and the retention time ranged from 14.15 to 23.13 during the analysis. The maximum retention time was registered in Heneicosanoic acid with 126 numbers of ions followed by Eicosanoic acid with 21.6 rt and 124 number of ions. The minimum retention time was registered in benzene with 14.15 rt and 92 number of ions during the analysis. 5-nonynoic acid (15.93 rt), Pentadecanoic acid (17.17 rt), Estra-1, 3, 5(10) Trien 17a-ol (17.92 rt), E,E,Z-1,3,12-Nonadecatriene-5,14-diol (18.85 rt), 6-Cyclooctylamino-5 (19.75 rt) and 4-Hexadecynoic acid (20.32 rt) recorded, respectively. The chromatogram of *Tecoma stans* leaf registered around six chemicals with highest retention time of 36.958 with the molecular weight of 161 [15]**.** And the compound was identified as boschniakine. A number of pyrindane alkaloids [16], tecomine [17] were isolated from the plants.

TABLE 1

GC-MS results of methanolic leaf extract of *Senna alata*

S.No	Phytochemicals	Retention time	No.of ions
1	Benzene	14.15	92
2	5-nonynoic acid	15.93	75
3	Pentadecanoic acid	17.17	158
4	Estra-1,3,5(10)-trien-17a-ol	17.92	168
5	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	18.85	226
6	6-Cyclooctylamino-5	19.75	160
7	4-Hexadecynoic acid	20.32	132
8	Heneicosanoic acid	23.13	126
9	Eicosanoic acid	21.6	124

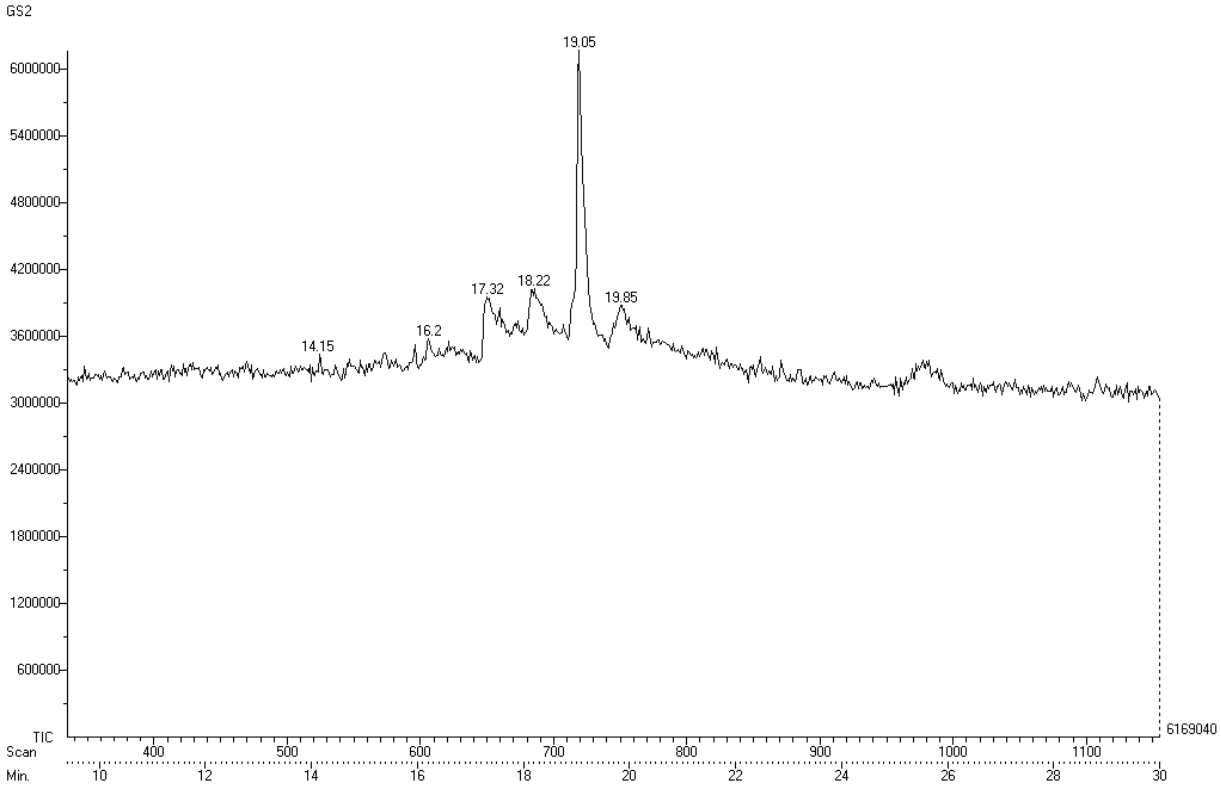
In the present investigations, the phytochemicals registered by the stem extract are tabulated in the Table.2. There were six phytochemicals recorded during the GC-MS analysis and almost every compound has a history of therapeutic values. Dasycarpidian-1-methanol registered with 19.85 rt and 223 number of ions, followed by phytol which recorded 19.05 rt and 166 number of ions the lowest retention time (14.15) was recorded by N-Ethyl-n-butan-4-ol-nitrosamine with 97 number of ions. The other compounds registered during the study were Trioxsalan, a-octodecan-12-ynoic acid and 14-hydroxy-14-methy hexadex-15-enoic acid which have a record for their medicinal value. About 19 and 31 phytochemical compounds were registered from Tagetes erecta leaf and flower extract recorded the maximum compounds due to high metabolic rate than any other parts of the plants[18].

TABLE 2

GC-MS results of methanolic stem extract of *Senna alata*

S.No	Phytochemicals	Retention time	No.of ions
1	Trioxsalen	16.2	128

2	9-octadecan-12-ynoib acid	17.32	200
3	14-hydroxy-14-methyl-hexadec-15-enoic acid	18.22	234
4	Phytol	19.05	166
5	Dasycarpidan-1-methanol	19.85	223
6	N-ethyl-n-butan-4-ol-nitrosamine	14.15	97



CONCLUSION

Senna alata has proved to have certain medicinal value in terms of the compounds which are registered during the GC-MS analysis of leaf and stem extracts during the present study. The account of this result, it is very keen to do further research on various aspects of biochemical investigations to prove the extract value of this plant species.

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