International Journal of Advance Research in Science and Engineering
Volume No.07, Special Issue No.03, April 2018
www.ijarse.com
IJARSE
ISSN: 2319-8354

WOUND HEALING MECHANISM IN FISH MUSCLE TISSUE AFTER ADMINISTRATION OF *Coscinium fenestratum, Azadirachta Indica, Cynodon dactylon* PLANT EXTRACT

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ABSTRACT

The Wound healing process on fish muscle and its repair mechanism is a natural process. The wound healing is achieved through four phases: Hemostasis, Inflammation, Proliferation, and Remodelling. The fish model taken for the wound healing process is Cyprinus rubrofuscus (Koi carp). The plants choosen for the administration in the wounded region are Coscinium fenestratum, Azadirachta Indica, and Cynodon dactylon. The chosen plant samples were analysed for the presence of alkaloids, flavonoids, tannins, terpenoids, glycosides, steroids, and saponins to confirm the phytochemicals in the plant extracts. The plants samples were then analysed with gas chromatography mass spectroscopy and the results obtained from GC-MS shows highest retention time (i.e RT=18.22) in Azadirachta Indica, for Coscinium fenestratum RT=16.66, and for Cynodon dactylon RT=16.68. From GC-MS results and the rate of wound healing Azadirachta Indica shows the best result.The sample analysed for the process of wound healing naturally show a decreasing in the size of the wound from day1 (1.12cm) to day7 (0.90cm). The collagen dermal patches with chosen plant extract were administrated to overcome the slowdown process of wound healing. The plant extract (A.indica) on administration shows an (38.8%); the plant extract (C.fenestratum) on administration shows an (29.4%); and the plant extract (C.dactylon) on administration shows an (27.5%) increased the rate of wound healing.

Keywords*: Azadirachta indica, Cyprinus rubrofuscus, Coscinium fenestratum,, Cynodon dactylon, gas chromatography-mass spectroscopy*

1 INTRODUCTION

The wound healing is a normal biological process in all living organisms and there are factors which affects the natural wound healing and slows down the process of wound healing (S. Guo *et al.*,). To overcome the slowdown wound healing process cause by external factors, dermal patches with plant extract are administrated on the wound surface which would protect the wounded area from external factors and also promote wound healing (S. Baar *et al.*,). Some of these plants have been screened scientifically for the evaluation of their wound healing activity in different pharmacological models (*Tuhin Kanti Biswas et al.*,). Collagen is a natural substrate for cellular attachment, growth and differentiation, and promotes cellular proliferation and differentiation. Nowadays, many commercial and experimental products have been introduced to improve cutaneous wound

healing (Zbigniew Ruszczak). Thus the collagen as dermal patch with plant extract was administrated for the wound healing in the fish and other models.

2 MATERIALS AND METHODS

For the present study, twenty number of *Cyprinus rubrofuscus* fishes were collected from lemuria fish farm near Madhavaram RedHills high road, Chennai (13.153684, 80.225790), Tamil Nadu, India. All the fishes collected was about 9-10cm in size and weighted 6-7grams. The fish are packed in a sterile plastic bag with pond water and transported to the college laboratory in 1hr and transferred to the glass tank. The plant samples *Coscinium fenestratum, Azadirachta indica* leaves, *Cynodon dactylon* were collected from Tharakshi Village (13.115425, 79.9130058), Uthukottai Taluk, Thiruvallur District, Tamil Nadu, India.

2.1 Acclimatization of fish: The fishes after collection was shifted to a fish tank which had an oxygen supply to it. The fish are kept in laboratory condition for the fish to get adapted to the environmental changes and pH of the water. The duration of the acclimatization was 15-17days. During the acclimatization period no fishes died. The fishes were fed twice a day and the water is changed on every two days. The source water was replaced with the college water in small units in order to provide time for acclimation to new temperature and water conditions.

2.2 Plant extraction for wound healing: Three plant samples were taken namely

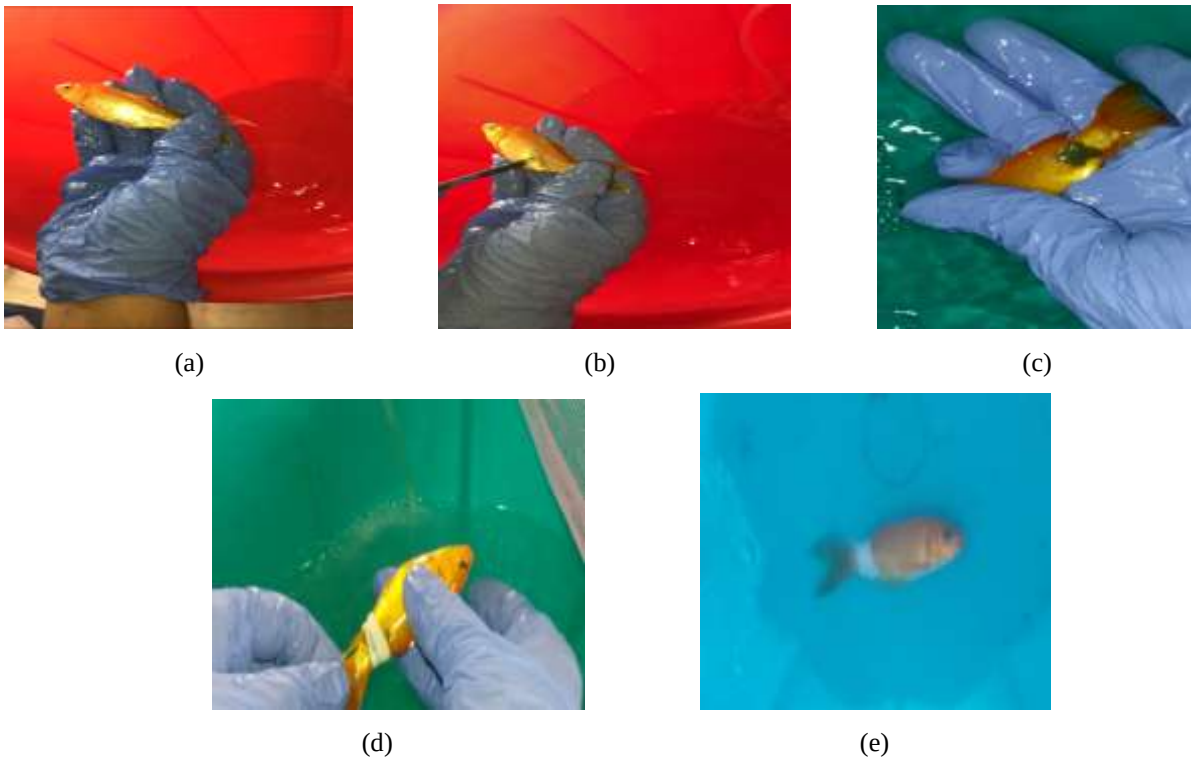
- *Coscinium fenestratum*
- *Azadirachta indica*
- *Cynodon dactylon*

For extraction and used for wound healing process in the fish sample.

The collected plant samples (Coscinium fenestratum, Azadirachta indica) were shade dried in the college laboratory for 10days until it completely dries and no moisture was present. The shade dried plant sample was grinded into powder with the help of motor and piston to obtain the powdered plant extract. The powdered samples of the plant were packed in a zip lock plastics so as to keep it free from moisture. The plant sample (Cynodon *dactylon*) was extracted with soxhlet apparatus. The extract collected was in the form of liquid which was stored in brown bottles for keep it protected from sunlight.

2.3 Experimental design for administration of plant sample in wound healing process: The experiment was carried out by first taking two tanks which was filled with one third of water. Eugenol was added in one of the tanks and double oxygen supplier was kept inside the water. The other tank was double oxygenated and kept ready to transfer the fishes after being sedated using eugenol. After sedation process, the fish were taken and wound was caused on the area near to the tail region. The plant extract was applied on the wound caused area and the wounded area was covered with the collagen patch. The step5 was repeated for the three chosen plant extract. After the completion of step5 the fishes were left in the double oxygenated eugenol free tank for the fish to come back to its normal stage after being sedated. During step7 period, the fish become little excited due to

the action of eugenol. Four fishes for each sample and a control was separated in different tanks and all the four batches were kept for observation in the laboratory under the acclimatization condition where it previously got adapted. The observations were done for 7days and the range of wound healing was observed, noted and the pictures of the wound were taken at intervals from day 1to day 7.



(a) (b) (c)

(d) (e)

Fig 1: (a) Handling of the fish during the experiment, (b) Developing wound to fish with a sterile knife, (c) Administration of the chosen plant on the wounded region, (d) Administration of the collagen dermal patch on the wound, (e) The fish left into the oxygenated water tank after administration of the chosen plant for the wound healing process

2.4 Phytochemical analysis of plant extract: The phytochemical analysis was done with the chosen plant extract to identify the presence of alkaloids, flavonoids, tannins, terpenoids, glycosides, steroids, and saponins. The screening procedures to identify the presence of these are as follows: screening of alkaloids in chosen plant- Extract of 1ml was taken and a drop of Hager's reagent was added along the sides of the test tube. Yellow precipitate indicates the presence of the alkaloids, screening of flavonoids in chosen plant- Extract of 1ml was taken and two drop of dilute sodium hydroxide was added. Appearance of yellow colour indicates the presence of flavonoids, screening of tannins in chosen plant- Extract of 2ml was taken, and 2 ml of 10% ferric chloride solution was added drop by drop. A blue black precipitate indicated the presence of tannin, screening of terpenoids in chosen plant- Extract of 5ml was taken, 2ml chloroform and few drops of conc.H_2SO_4 is added to form a layer. A reddish brown colour indicated the presence of terpenoids, screening of glycosides in chosen plant- Extract of 1ml was taken in a test tube. Then 1ml of H_2SO_4 was added. It was observed for the formation of red colour, screening of steroids in chosen plant- Two ml of chloroform and concentrated H_2SO_4 were added

with the 5ml of aqueous plant extract. In the lower chloroform layer red colour appear that indicates the presence of steroids, and screening of saponins in chosen plant- Extract of 1ml was taken, 20ml of distilled water and agitated in graduated cylinder for 15mins. Formation of soap layer indicates the presence of saponins.

3 RESULTS AND DISCUSSION

In this study of wound healing on the fish muscle tissue on administration with the plant extract shows us the result of wound healing. The comparative study between the three plants was done on the fish muscle tissue and the following results are obtained. The plants samples chosen are *Coscinium fenestratum, Azadirachta Indica,* and *Cynodon dactylon* these plant extracts have properties of wound healing in the fish muscle. The three plant samples analysed for the presence of the volatile compound by gas chromatography mass spectroscopy and the results are of gas chromatography were given in the below graphical representations. After the wound was caused, the collagen patch was applied with the plant extract and the observations were taken on day 1, day 3, day 5, and day 7 intervals. The image of wound healing on the fish muscle tissue was taken and the lengths of the wound were measured at the respective day with vernier calliper and the measurements were tabulated at regular intervals. The phytochemical analysis was done by various methods to show the presence or absence of the alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and terpenoids in the three plant extracts and the results were tabulated.

3.1 Phytochemical analysis: The phytochemical analysis was performed and results are shown in table 1. Subrata kumar *et al.*2011 studied the presence of phytochemical in various plant samples and identified the presence or absence in the selected plant sample.

Table 1: phytochemical screening tabulation for the chosen plant extract

S.NO	**NAME OF THE PHYTOCHEMICAL**	*A.indica*	*C.fenestratum*	*C.dactylon*
1	Alkaloids	Positive	Positive	Positive
2	Flavonoids	Negative	Positive	Positive
3	Tannins	Positive	Positive	Positive
4	Saponins	Negative	Positive	Positive
5	Glycosides	Positive	Positive	Positive
6	Steroids	Positive	Positive	Positive
7	Terpenoids	Positive	Negative	Positive

Present= positive, absent= negative

The phytochemical screening was done on *A.indica, C.fenestratum* and *C.dactylon.* The major phytochemical were present in the C.*dactylon* which shows positive results for every phytochemical screening test.

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www.ijarse.com
IJARSE
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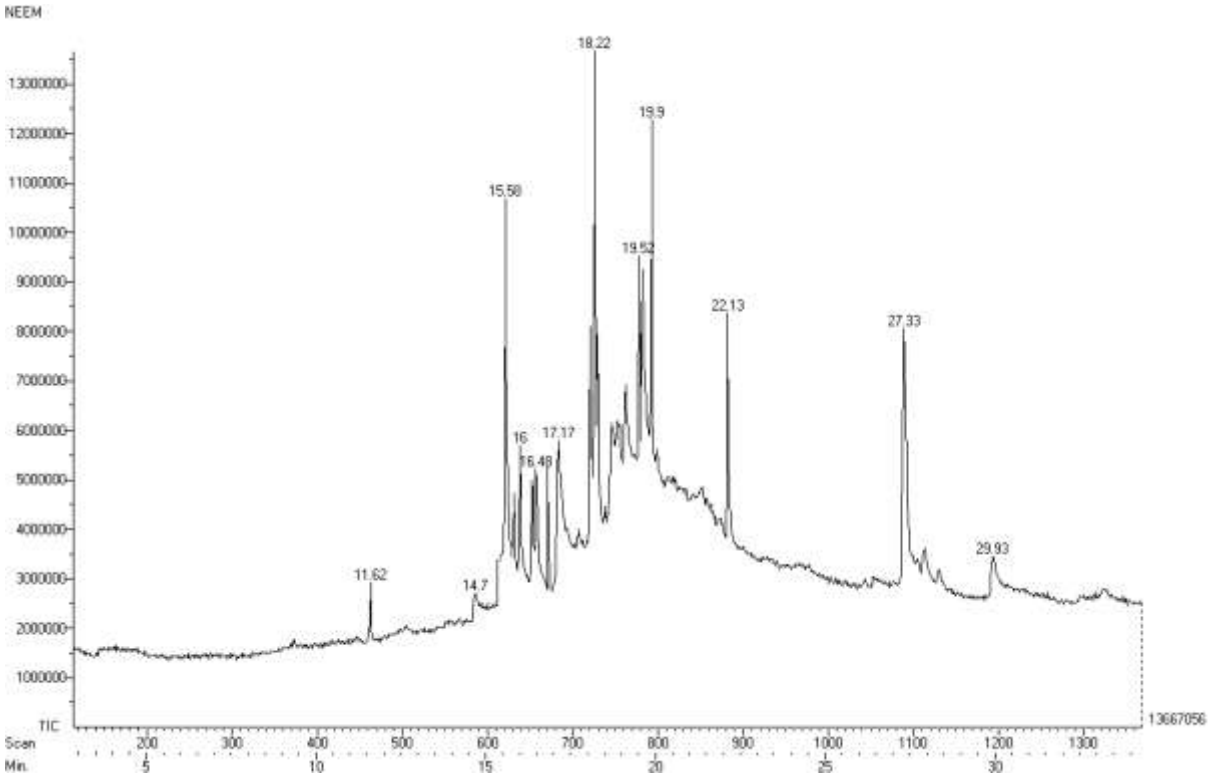
3.2 Analysis of wound healing: The wound length was measured to know the percentage difference in the wound being healed after administration of the dermal patch with chosen plant extracts. The measurement was done with vernier calliper and its shown in table 2.

Table 2: Analysis of the wound at regular interval

Plant sample	DAY1	DAY3	DAY5	DAY7
A.indica	1.12cm	1.06cm	1.01cm	0.90cm
C.dactylon	1.11cm	1.09cm	1.03cm	0.98cm
C.fenestratum	1.11cm	1.08cm	1.03cm	0.99cm

3.3 GC-MS result of the plant extract

3.3.1 GC-MS of *Azadirachta indica*



International Journal of Advance Research in Science and Engineering
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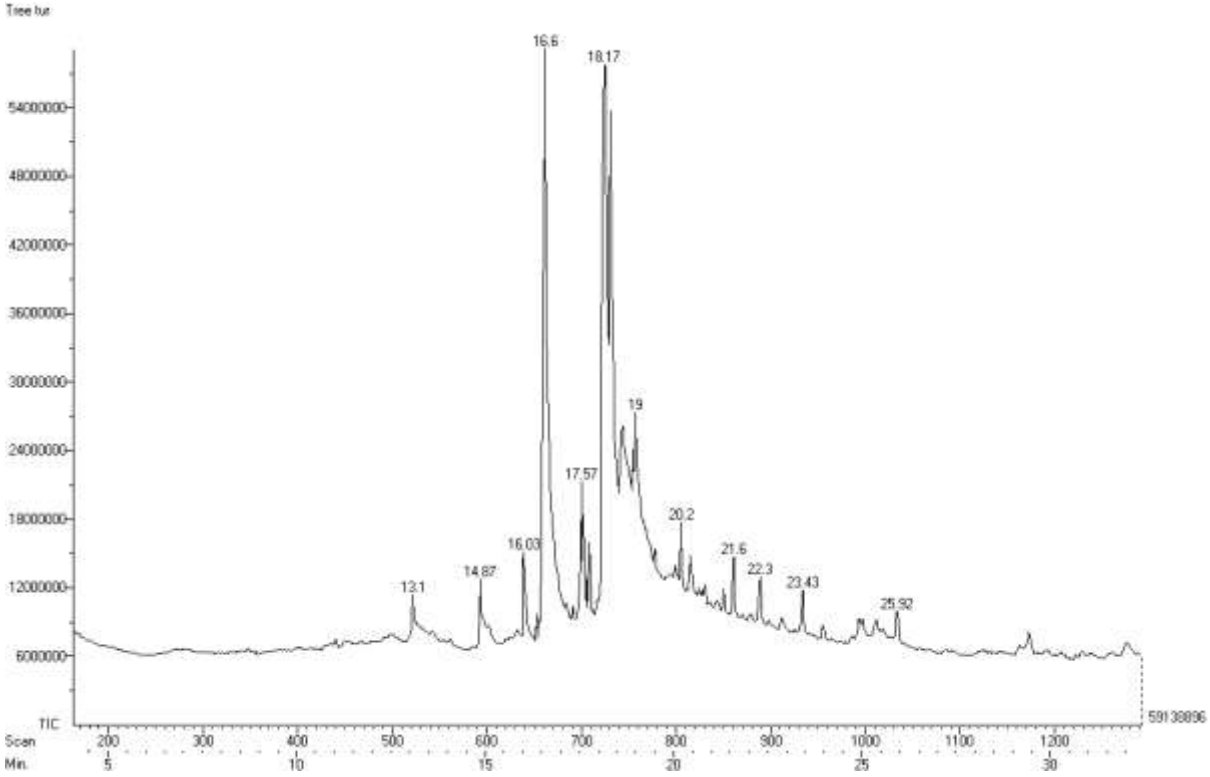
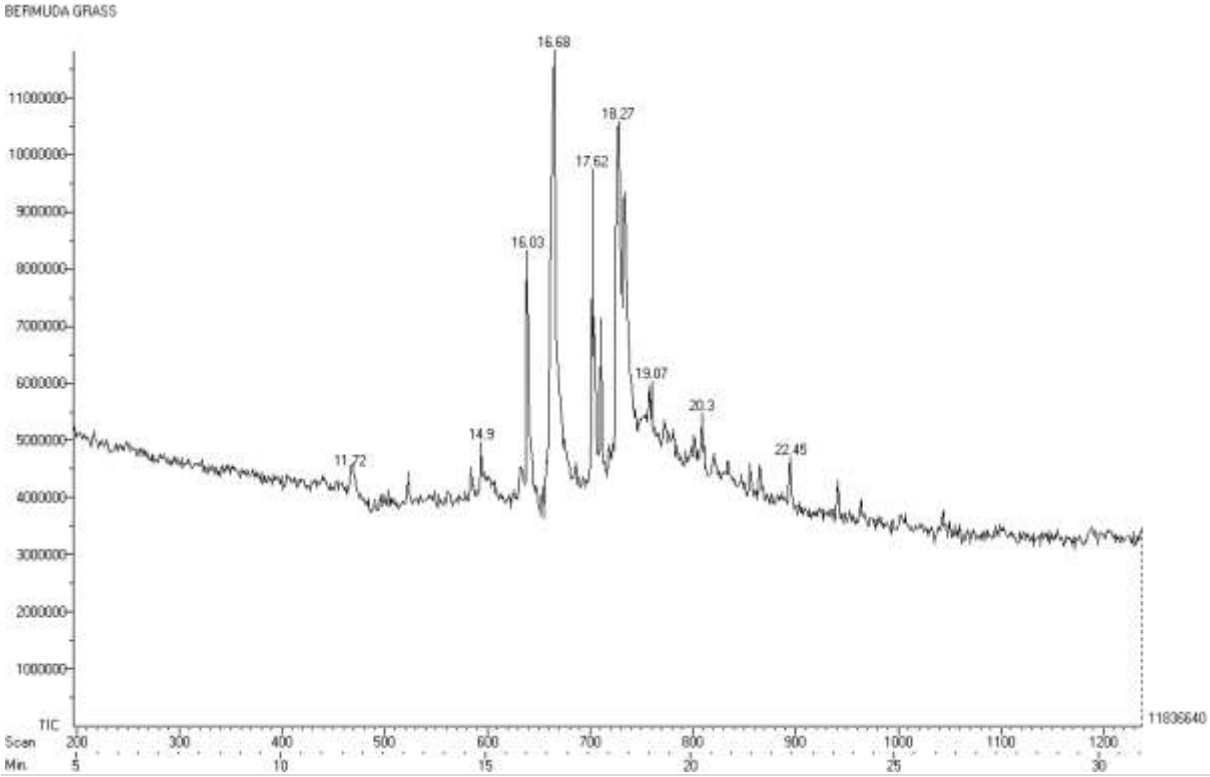
www.ijarse.com
IJARSE
ISSN: 2319-8354

3.3.2 GC-MS of *Coscinium fenestratum*



3.3.3 GC-MS of *Cynodon dactylon*



From the GC-MS study it was found that many different compounds are present in the chosen plant extract at different retention time intervals. The peaks show the presence of the volatile compound in the chosen plant samples.

4 CONCLUSIONS:

Based on the results obtained the plant extract (*Azadirachta Indica*) on administration shows an (38.8%) increased rate of wound healing; the plant extract (*Coscinium fenestratum*) on administration shows an (29.4%) increased rate of wound healing; and the plant extract (*Cynodon* dactylon) on administration shows an (27.5%) increased rate of wound healing. The results obtained from GC-MS shows highest retention time (i.e. RT=18.22) is showed in *Azadirachta Indica*, for *Coscinium fenestratum RT=16.66,* and for *Cynodon dactylon RT=16.68.* The compound which was present in this highest retention time are Nonadecane, 5-Methyl-7-Phenyl-1,3-diazaadamantan-6-one hydrazine, and n-Hexadecanoic acid in the respective chosen plant extracts.

After the observation of the wound healing from day1 to day7 it was found that the results shown by *Azadirachta Indica* has highest wound healing properties than *Coscinium fenestratum and Cynodon dactylon.*

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