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National Symposium
On

Applied Zoology, Profitable Animal Production, and Health: Current Status and Future Progress (NSAZ-2022)

Organized by

Department of Zoology and Fishery Science, Rajarshi Shahu Mahavidyalaya (Autonomous), Latur- 413531, Maharashtra

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Assessment of antibacterial potential of formulated ointment containing medicinal plants against some fish bacterial pathogens.

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ABSTRACT:

In present investigation the ointment is made with *Holarrhena pubescens*, *Cocculushirsutus*, *Ocimum tenuiflorum*, ;Cross streak technique shows inhibition zone greater than 10 mm in ointment C followed by B, D, A and E against all the test pathogens. By good quality of its greater inhibition zone in the cross streak method and positive antibacterial activity, ointment C (*Holarrhena pubescens* 3.5 %) was selected for further experiments. the test were carried up to 4 week and zone of inhibition were checked at 7^{th} , 14^{th} , 21^{st} , 28^{th} day .the greater inhibition zone found in ointment C for *Enterobacter aerogenes it were shown* (16.9 ± 0.5) followed by ointment D (14.3 ± 0.5) at 21^{st} day. the greater inhibition zone found in ointment C for *Aeromonashydrophila it were shown* (17.9 ± 0.4) followed by ointment D (15.3 ± 0.6) at 21^{st} day. Ointment E which do not contain medicinal plat mix do not shows greater inhibition activity .which clearly shows that present formulation responsible to inhibit bacterialgrowth infected to animals.

KEYWORDS: Medicinal Plants, Fish Health. Antibacterial.

Introduction:

Skin care measures are premeditated to apply when on the skin. some important measures are formulated creams, ointments and lotions which been used in the treatment of animal wound, fungal/bacterial infection bearing in mind the attentiveness of medicine with degree of solubility, current formulations are proved be beneficial for treating localized skin infection [1]Aquaculture is the fastest growing food-producing sector in the world, with an average annual growth rate of 8.9% and practiced in a variety of agro-climatic zones ranging from tropical to temperate area It includes farming of aquatic organisms, including fish, molluscs, crustaceans and aquatic plants. The world aquaculture has grown tremendously during the last 50 years from a production of less than a million tonnes to 59.4MT. The production in aquaculture is hampered by disease caused by various fish pathogens and is constraint to the culture of many aquatic species. The persistent disease problems in aquaculture necessitate the use of bacterial control agents as probiotics which can be effectively used as an alternative to antibiotics

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Plants having medicinal ability was been used as conventional medicine since the decades. The medicinal plant extract obtained from various parts viz. leaves, roots and stem have been utilized as ailments for many diseases [2] hence these all medicinal Plants used as necessary foundations in therapeutic mediators to cure various diseases occurred in human and animal. Conventionally the herbal medications served as holistic loom for protection and to improve metabolism and health [3]

Recent herbal medical ointment formulation hasbeen provided good result. Medicinal plants are significant foundations of novel chemical essences which work as helpful therapeutic results. Many investigations carried out in terms of wound healing treatment by using medicinal plants, current investigations were carried out on A.sessilis, M.citrifolia, Lycopodium Catharanthus, Euphorbia Wound is further been explained by WHS (Wound Healing Society), wound is physical damage which further consequences into break of the skin and open it so that it cause disorder related to regular skin composition and function [4] Consequencein the hammering of permanence of epithelial tissues. Unhealed injuries steadily create inflammatory arbitrators which cause pain and bulge at the wound site. [5] In present investigation we were used following plants having medicinal properties.

Medicinal Pants Used In experiment:

1. Holarrhena pubescens:

Commonly known as Indra-jao in regional language. A kind of shrub, grow up to 3 meter in height. Small stem with pale bark.. White color flowers, at the end of branches. This medicinal plant was been described within Ayurveda, use for Diarrhoea and stomach disturbances further the bark is helpful for piles, various skin diseases. *Cocculus hirsutus* (Vasanvel): *Cocculus hirsutus* most well-liked alongwith its medicinal use in asia, it is abandon source of various alkaloids, very few attempts have been made in terms of its antifungal and wound healing potential. *Ocimum tenuiflorum* (Rantulas): the medicinal use of *Ocimum tenuiflorum* is well known many studies carried out *in vitro*, on animal and human shows its unique property as Antimicrobial, antioxidant, anti-cataract, anti-inflammatory, radio- defensive, hepato-defensive, immunomodulatory, memory improvement, opposed to-asthmatic, diaphoretic, defiant to thyroid, useful in fertility, cure ulcer, opposed to emetic, opposed to-spasmodic, opposed to-leukodermal and helful in blood coagulant activities. [6,7,8,9]

Materials and methods:

II.1. Sample Collections:





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The fresh plant materials were collected from Local farms ad hills of Osmanabad The sample was identified and confirmed by using botanical keys of identification. The leaves of medicinal plants were dried and used dry powder of medicinal plants in variable amount (Table 1, Figure .A) as *Holarrhena pubescens* (indrajav), *Cocculushirsutus* (vasanvel) ,*Ocimum tenuiflorum* (rantulas) . The dehydrated plant leaves were crushed by a blender to get smaller particles.

II.2. Ointment Formulation:

Semisolid emulsions (O/W) oil in-water An emulsifying agent is used to scatter the aqueous stage to oily stage or vice versa [10]. The important components in cream formulation are emulsifying agent, water, oil, thickener and preservatives. The dehydrated sample was stored in the refrigerator at temperature 4 °C. To make an aqueous extraction Mix Medicinal Plants leaf were marinated in 2000 ml of water further it allow to heat (50 °C to 80 °C) constant stirring using magnetic stirrer.

Filtered and the dehydrated sample was stored in the refrigerator at temperature 2 °C. Herbal ointment was organized spitted by addition of oil fraction to aqueous fraction. Further it was mixed and thawed inside container stirring were applied continuously.

As soon as ingredients of medicinal plants were melted allowed for cooling process. During making of aqueous ointment 35% ointment was mixed with 65% of heated water. Sudden melting and cooling were obtained, chloroscresol used as preservative active ingredients of the plants extracts with dissimilar concentration of Holarrhena Pubescens (Indrajav)=2.5gm, 3gm, 3.5gm. 4gm were used. Physicochemical properties of mix medicinal plants and ointment were checked as per standard.

II.3. Study of Antagonistic action of ointment and wound healing

In present investigation the antagonistic action of the ointment tested with the help of Cross Streak Method suggested by Lechevalier and Waksman [11] The solitary streak of the chosen bacteria was prepared upon nutrient agar plate further incubated(37°). Pathogenic bacteria such as, *Enterobacter aerogenes Aeromonas*. *hydrophila* streaked to right side of original streak record of ribbon like band around it were noted. plates incubated (28°C) record taken of

inhibitory zones created by each isolates the measurement were also recorded as sensitive, moderate and resistant. One control plate was maintained.

Results:





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III.1: Antagonistic activity

Table 2: Antibacterial activity of the medicinal plant mix ointment against pathogens

| Pathogenic | Day | Ointment | Ointment B | Ointment C | Ointment D | Ointment |
|----------------|-----|---------------|------------|------------|------------|---------------|
| Bacteria | | A | | | | \mathbf{E} |
| | | | | | | |
| | 7 | 5.3±0.7 | 5.4±0.7 | 6.6±0.7 | 6.2±0. 1 | 4.1 ±0.1 |
| Vibrio | 14 | 5.9±0.4 | 5.9±0.5 | 6.9±0.2 | 7.2±0. 1 | 4.8 ± 0.1 |
| | 21 | | | | | |
| parahaemolytic | | 9.3 ± 0.2 | 9.8 ±1.5 | 16.9 ±0.5 | 14.3 ±0.5 | 6.4 ± 0.4 |
| us | 28 | | | | | |
| | | 9.2 ± 0.1 | 9.4 ±0.5 | 14.9 ±0.5 | 13.2 ±0.5 | 6.1 ± 0.3 |
| | 7 | 4.2±0.4 | 4.4±0.3 | 5.9±0.4 | 6.1 ±0. 3 | 4.3 ±0.2 |
| Aeromonas | 14 | 6.3±0.4 | 6.5±0.4 | 6.8±0.5 | 7.6 ±0. 5 | 5.0 ± 0.2 |
| hydrophila | 21 | 9.6± 0.4 | 10.1 ±0.5 | 17.9 ±0.4 | 15.3 ±0.6 | 6.1 ±0.8 |
| | 28 | 9.4 ± 0.5 | 9.9 ±0.5 | 13.9 ±0.6 | 12.9 ±0.5 | 5.1 ±0.6 |

The ointment formulations were O/W (oil water) type emulsion ointment. All A, B, C, D and E ointments showed good stability. The pH of ointments were ranges 6.2 to - 6.9 this ointment pH is neutral to the skin. These parameters were steady for 1 month. Cross streak technique shows inhibition zone greater than 10 mm in ointment C followed by B, D, A and E against all the test pathogens. By good quality of its greater inhibition zone in the cross streak method and positive antibacterial activity, ointment C (*Holarrhena pubescens* 3.5 %) was selected for further Experiments. the test were carried up to 4 week and zone of inhibition were checked at 7^{th} , 14^{th} , 21^{st} , 28^{th} day the greater inhibition zone found in ointment C for *Enterobacter aerogenes it were shown* (16.9 ±0.5) followed by ointment D (14.3 ±0.5) at 21^{st} day, the greater inhibition zone found in ointment C for *Aeromonashydrophila it were shown* (17.9 ±0.4) followed by ointment D (15.3 ±0.6) at 21^{st} day. Ointment E which do not contain medicinal plat mix do not shows greater inhibition activity which clearly shows that present formulation responsible to inhibit bacterial growth infected to animals.(Table 2)

IV-Discussion:

The major aim to formulate present ointment is for animals suffer from wounds .and most of time they use to treat it with mouth and chemicals can go in stomach which little bit cause





digestive disturbances. In present ointment most of plants are natural, so no major harm in physiology, test on animals will be further carried out, here we just put the results of antibacterial potential of present ointment formulation. The formulated ointment was oil in water type hence comfortable to wash out with water easily

Similar experiment was carried out using methanolic extract by using medicinal plant *Eucalyptus* anti-dermatopytic ointment were prepared [12] (Moghimipour *et al.*, 2009). pH is also in satisfactory limit. Lambers *et al.* (2006) [13] said that pH of skin is acidic but with wide series ranges 4-7. Spreadability is good in present ointment. Here at ointment C the best results are seen by variable percentage of medicinal plant mix. Similarly ointment formulated with single medicinal plant *Mangifera indica* also shown safe and best results [14]

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