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Isolation and Characterisation of bacteria from herbal tablets used in the treatment of *Rheumatoid arthritis*

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***ABSTRACT*:** Ayurveda is the science of life, a natural healing system developed by ancient Indians. Ayurvedic treatment includes herbs, diet, exercise, meditation, massage, exposure to sunlight, controlled breathing, and yoga. *Rheumatoid arthritis* (RA) is an autoimmune disease where the body's immune system mistakenly attacks the synovium, a thin membrane that lines the joints. Two different samples of herbal medicinal tablets, of two different manufacturers, were collected that are used in the treatment of RA from the medical store and brought to the laboratory. They were coded as YG and AR and stored at room temperature until use.These samples were dissoved in sterilizrd distilled water and diluted by Serial dilution method and randomly selected 10 samples were spread on sterile nutrient agar plates. These ten samples shows microbial growth and all were found to be gram-positive constituting 3 rod-shaped and remaining seven were cocci shaped. Cultural, morphological and biochemical studies resulted that all ten isolates differ from one another. Both the herbal medicinal tablet samples were used in treatment of RA contained measurable number of bacteria and should be purified before use.

***KEYWORDS*:** Ayurveda, *Rheumatoid arthritis*, Autoimmune disease.

***INTRODUCTION*:** Ayurveda is the science of life, a natural healing system developed in India. Ayurvedic treatments include diet, exercise, meditation, herbs, massage, exposure to sunlight, controlled berating, and yoga it is the oldest system of medicine (Hitokoto et al., 1978). The object of Ayurveda is to counteract the imbalance of three essential physical elements viz. Vata, Pitta, and Kapha which constitute the Tridosh from which the body originates. The environment and the quality of the raw materials employed in the manufacturing of medications have an impact on their microbiological quality some infections. The usage of highly polluted natural raw materials has been linked to outbreaks (Enayatifard et al 2010).

Rheumatoid arthritis (RA) is an autoimmune disease where the body's immune system mistakenly attacks the synovium, a thin membrane than lines the joints (Firestein, 2003). It causes chronic inflammation that leads to pain, swelling, and stiffness. Eventually, bones and joints can be damaged, leading to disability. RA usually affects joints on both sides of the body equally meaning if a joint on 1 side is affected, the same joint on the opposite side is affected.

Wrists, fingers, knees, feet, ankles, and elbow are most often affected body organ. RA can also affect other organs, and people who have RA are at higher risk for other disease including heart disease, diabetes, and osteoporosis (McInnes et al 2008).

MATERIALS AND METHODS:

1. Collection of Samples-

Two different samples of herbal medicinal tablets used in treatment of RA from different manufacture were collected from the market as sealed packed and in bottles then brought to the laboratory and coded as YG and AR and stored at room temperature until use.

2. Enumeration of Bacteria From Herbal Medicinal Tablets-

Serial dilutions of each sample were prepared using serial dilution technique (Booth 2006). 0.1 ml of each dilution was spread on a sterile nutrient agar plate. With the help of alcohol and a spreader. Then these plates were incubated at room temperature for 48 hrs. after incubation, colonies were observed and the enumeration of organisms were done by using the formula given by Pochampally R. (2008) as colony forming units (cfu/g/ml) of sample = number of colonies x dilution factor x 10

3. Isolation of Bacteria From Herbal Medicinal Tablets-

Using four quadrant streak plate method (Sanders, E.R. 2012) the isolation of different bacteria from the sample was done using a sterile nutrient agar plate and incubation at room temperature for 48hrs. The colonies developed of different morphologies that were selected and coded for further work. The morphological character of each colony was studied and recorded. All the isolates were preserved at refrigeration temperature on a sterile nutrient agar slant and after every two months, reinoculated the isolated organisms and that were maintained in the laboratory. Then the gram nature of the isolated organisms was observed by, Hucker and Conn (1923) modified gram staining method. The motility of isolates was observed by the Hanging drop technique.

RESULT AND DISCUSSION:

For enumeration of microbial colony forming units (cfu) two samples of two different manufacturers were screened that were used on treatment of RA. These two samples were collected from local medical store and named as YG and AR randomly. These two samples were dissolved in sterilized distilled water and diluted by serial dilution method (Booth 2006). A total twenty different diluted samples were obtained, from which ten randomly selected saples six from YG viz. YG-5, YG-7, YG-8, YG-9, YG-10, and YG- 11 and four from AR viz. AR-1, AR-2, AR-3, and AR-7 were selected for isolation and characterization of bacterias. These ten samples were inoculated on nutrient agar plate and incubated at room temperature under sterilized condition.

The results of inoculated saples were estimated using formula given by Pochampally R. (2008) for colony forming units (cfu). The average cfu was recored as $31.094x10^8$ and

$27.925x10^8$ cfu/g/ml for YG and AR respectively. It means that all the randomly selected test samples were contaminated with measurable number of bacteria. These products have the potential of contamination with different microorganisms. This is due to raw materials contamination and unhygienic production conditions (Enayatifard et al 2010).

We had also examined effect of temperature on growth of ten test samples on bacterial growth and it is represented in table no. 1. We had reported that AR-2 only shows growth at 10^0c remaining nine test samples were filed to grow at 10^0c, while all the ten test samples viz. YG-5, YG-7, YG-9 YG-10, YG-11 AR-1 AR-2, AR-3, and AR-7 were positively responed to 30^0c and showed good growth, similarly for 50^0c. Contradictory results were recorded for 70^0c in which not a single test sample showed there growth response and failed to grow at this temperature. So, from ths we can counclude that for bacterial growth temperature play a vital role and bateria can not survive very less as well as very high temperature.

The response of test samples to pH level were also recorded. pH -2 and pH-4 did not showed any growth response of any test samples but for pH-6 five test samples showed there growth viz. YG-11, AR-1, AR-2, AR-3, and AR-7 and for pH-10 all the ten samples showed there growth. These findings demonstrated that raw material of natural origin in these tablets has some initial microbial levels of contaminants related to herbal tablets used in the treatment of RA (Enayatifard et al 2010).

Sr. no.	**Code of isolates**	**Temp. 10^0c**	**Temp 37^0c**	**Temp. 50^0c**	**Temp. 70^0c**	**pH-2**	**pH-4**	**pH-6**	**pH-8**	**pH-10**
1	YG-5	-	+	+	-	-	-	-	+	+
2	YG-7	-	+	+	-	-	-	-	+	+
3	YG-8	-	+	+	-	-	-	-	+	+
4	YG-9	-	+	+	-	-	-	-	+	+
5	YG-10	-	+	+	-	-	-	-	+	+
6	YG-11	-	+	+	-	-	-	+	+	+
7	AR-1	-	+	+	-	-	-	+	+	+
8	AR-2	+	+	+	-	-	-	+	+	+
9	AR-3	-	+	+	-	-	-	+	+	+
10	AR-7	-	+	+	-	-	-	+	+	+

Table no. 1. Growth of different isolates at various temp and pH values

+ = Growth - = No growth

CONCLUSION:

All the two samples of herbal medicinal tablets used in the treatment of *Rheumatoid arthritis*, which were collected from the market show the presence of a variable of bacteria and varieties of bacteria were associated with these formulations. It was observed that both herbal tablets contained a measurable number of bacteria. Bacteria isolated from these tablets may

adversely affect the tablets due to their metabolic activities. All the samples of herbal drugs evaluated did not generally meet the standards for microbial limits as specified in official monographs. Such samples can adversaly affect health status of consumers as well as the stability of the products.

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