

# *Summary*

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Domestic animals with skin infection were selected from different geographical regions. Skin scrapings and hair plucks were collected and placed on the Dermatophytes Test medium (DTM). Dermatophytes grown on this medium were isolated and cultured separately. Fungal pathogens were cultured on Sabouraud Dextrose Agar medium (SDA) and bacterial pathogens on Muller Hinton Agar medium (MHA). The isolated pathogens were identified by their morphology and by biochemical test at Animal disease Intelligent Unit (AIDU), Madurai. The fungal pathogens identified were *Aspergillus niger*, *Candida albicans*, *Curvularia geniculata*, *Geotrichum candidum*, *Microsporum gypseum*, *Penicillium sps*, *Rhizopus rhizopodoformi*, *Rhodotorula minuta*, *Trichophyton mentagrophytes* and bacterial pathogens were *Staphylococcus sp* and *Streptococcus sp*.

Nine plants *Allium sativum*, *Andrographis paniculata*, *Annona squamosa*, *Azardirachta indica*, *Corallocarpus epigeaus*, *Lawsonia inermis*, *Madhuca longifolia*, *Ocimum sanctum* and *Pongamia pinnata* were screened for their antimicrobial properties against the selected pathogens. Different solvent extracts like petroleum ether, benzene, chloroform, acetone, methanol and water were used for the study. Among the plants tested *Andrographis paniculata*, *Lawsonia inermis* and *Madhuca longifolia* were found to exhibit good inhibitory effect against the selected pathogens. Hence, these three plants were selected for further studies.

Physico-chemical, extractive value, fluorescence analysis and preliminary phytochemical studies were carried out in the selected three plant. Chloroform,

methanol and water extracts confirmed the presence of most of the secondary metabolites.

Methanol extract showed the presence of the major active compound Andrographolide in *A.paniculata* and Lawsone in *L.inermis* along with different unknown compounds. In TLC studies, andrographolide was detected at the Rf value of 0.53 and lawsone was detected at the Rf value of 0.73. In HPLC studies, andrographolide was detected at the retention time of 2.5 minutes and lawsone at 2.9 minutes.

Influence of age, geographical variation and seasons on the quantity of andrographolide and lawsone were studied. Andrographolide content was higher in 120 days old plant. Lawsone and andrographolide content were found to be higher in the plant collected from pasumalai hills than plains. The quantity of Lawsone content was found to be more in *L.inermis* collected during the month of july – august.

Scanning Electron Microscope studies of the selected plant powders showed the presence of nano particles of various sizes under 80X-5000X magnification.

Antioxidant studies were carried out in the selected plant materials by reducing power assay method. Methanol and water extracts were used for the study. Ascorbic acid used as a standard reference. Methanol extracts exhibited higher antioxidant property than water extract. However, pure compounds andrographolide and lawsone exhibited higher antioxidant property than plant extracts. Among the plant extracts used *M.longifolia* exhibited highest antioxidant property.

*In vitro* studies were carried out to test the efficacy of the plant extracts in killing the pathogens isolated from infected animals. Different concentrations (20-100µg) and combinations 1:1 and 1:1:1 of methanol and water extracts of plant materials were used for the study. Minimum Inhibitory Concentration of the

extracts were also determined. Among all the trials conducted, combination of three plant extracts showed good inhibitory effect than other combinations.

Antifungal drugs like povidone iodine (100µg), candid cream (100µg), ketoconazole (100 µg) and itraconazole (100µg) were used as positive control for fungi and ampicillin (100µg) used as a positive control for bacteria. Pure methanol used as a negative control for both fungal and bacterial pathogens. Comparing the result, plant extracts exhibited good antimicrobial effect than allopathic drugs used.

*In vivo* studies were done on infected cows using the combination of three plant extracts (1:1:1). Allopathic drug Povidone iodine and curables were used as control. Animals were grouped into three groups as group A, B and C. Group A animals were treated with plant extracts, group B and C were treated with povidone iodine and candid cream respectively. Animals (Group A) treated with plant extracts recovered completely within 5-8 days at the higher concentration of 400-500µg whereas animals treated with allopathic drugs (group B and C) took 19-21 days for complete recovery.

After standardization of dosage from *in vivo* studies, herbal formulations were prepared following the traditional and industrial (pharmaceutical) methods. Fresh plant extracts were used for the preparation of ointments. To check the quality of the ointments, different parameters like pH, homogeneity, viscosity, spreadability, stability and primary skin irritation tests were evaluated in both the ointments. The parameters evaluated were found to be ideal for standard quality drug as prescribed in siddha pharmacopeia.

Heavy metals studies were carried out in plant materials along with soil samples collected from their habitat and herbal ointments for the accumulation of heavy metals like lead, chromium, nickel and cadmium using Atomic Absorption Spectrometer. In all the samples tested, it was found to be present below the

permissible level as set by WHO. Thus, the ointment is proved to be safe to treat skin diseases.

Clinical trials were conducted on infected domestic animals like cows, dogs, goat, sheep, horse and ox. Treatment with herbal ointments were given after receiving consent from the animal owners. The infected animals were identified based on their symptoms and treated with ointments. Animals from different geographical regions, age and breed were grouped as mildly infected (+), moderately infected (++) and severely infected (+++). The observations showed that, when traditionally prepared ointment was applied, animals with mild infection took 5-6 days, with moderate infection took 6-8 days and with severe infection took 9-12 days for complete recovery. Whereas an industrially prepared ointment was applied to mildly infected animals it took 6-7 days, moderately infected took 7-8 days and severely infected took 8-12 days for complete recovery. To compare the efficacy of herbal formulations allopathic drugs povidone iodine, candid cream and curable ointment were used as control. Povidone iodine took 17 days whereas candid cream and curable took 19 days to cure the disease.

Thus, from the present investigations it is proved that herbal formulations are more effective to cure the skin disease than allopathic drugs used. The toxicological studies proved that herbal ointments were devoid of toxic elements. Hence, the herbal ointment can be recommended for treating the skin disease on domestic animals.