Discussion

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Medicinal herbs are the potential source of therapeutic aids. They have a significant role in maintaining the health system of human beings as well as animals all over the world (Moreki, 2013). The World Health Organization (WHO) has recognized the inevitability of the use of alternate system of medicine for certain conditions such as cancer, skin diseases, multiple sclerosis, etc., for which no definite solutions are available in allopathic system of medicine (Anonymous,1992).

Ethnoveterinary medicine is the holistic interdisciplinary study of the local knowledge, social culture and environment associated with animal healthcare and treatment. It is less systematic, less formalized and not universally recognized as a valid method of disease control in animals. Yet traditional veterinary medicine is very important in developing countries where conventional remedies for animal health care are inaccessible or unaffordable to poor rural farmers (McGaw *et. al.,* 2008). The traditional knowledge of ethnoveterinary specialists and local healers are knowledgeable and is experienced but their knowledge are not documented and is rapidly ruining (Jain 1999). Therefore, it is important to standardize and document the ethnoveterinary medicinal plants and practices.

Skin diseases are the most common disease caused by fungal pathogens worldwide and are considered to be a major public health problem (Shinkafi and Manga, 2011). It is mainly caused by fungal organisms like *Microsporum, Trichophyton* and *Epidermophyton* (Nopamart Trankranrungsie, 2011). They grow best in warm and humid environments and are therefore more common in tropical and subtropical regions (Blanka havlickova *et al.,* 2008). *Microsporum gypseum*

and *Trichophyton mentagrophytes* are considered to be the main causative pathogens of skin infections. Although, it is a superficial skin infection on cattle, it has a significant impact on management and economic status. Once the disease occur in a herd, it spreads easily from one animal to another. Spores may survive in the environment for 2 to 3 years. Animals introduced from other places into the herd are likely to come into contact with infection. Beyond fungal infections, secondary bacterial infections will also occur in skin diseases (Gudding and Lund, 1995). These dermatophytes invade skin, hair and nail and cause dermatophytosis. Infection usually begins in growing hair or the stratum corneum of the skin. The lesions are characterized alopecia, scaling, crusts, erythema and pruritus of varying degrees. Young animals are affected very often and are particularly common in cold climates where animals are stabled for long period of time. In cattle, ringworm is seen most often during winter season. Infections are rare in sheep and goats (Hainer, 2003)

Practically, the antifungals available in the market are the same as those developed for the human but with modified strengths (Hector, 2005). Few antifungal agents are available and licensed for use in veterinary treatment. The use of these drugs is limited to treat animal due to their high toxicity, cost effectiveness and problems of chemical residues in products (Araujo *et al.*, 2009). Moreover most of the antifungal drugs are immuno suppressive and cause many side effects like fatigue, blurred vision, hepatotoxicity, hair loss, deposition of drug residue in milk, etc., (Good man and Gil man, 1995).

In recent years, number of effective, allopathic antifungal drugs like terbinafine, itraconazole, fluconazole, griseofulvin, enilconazole etc., are developed. Enilconazole (Imaverol) is licensed for use in dogs and should be applied every three days, in conjunction with systemic therapy. Fatal idiosyncratic reactions have been reported in animals treated with enilconazole (Ranganathan et

al, 1997). The expense burden from continuingly rising prices of several conventional antifungals is therefore inevitable. In this regard, the re-emerging interest of natural products, including plant-derived antifungals, could provide a niche for herbal formulations against dermatophytosis in animals with possible better affordability (Trakranrungsie *et al.*, 2006).

Thus, all these drugs are costly and cause serious side effects. Hence, it has become necessary to identify and develop novel compounds of plant origin for treatment of skin disease on animals. The potential role of plants or plants extracts as sources for new antifungal drugs has never been more apparent (Nopamart Trakranrungsie, 2011). Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicines (Venugopal Pankajalakshmi and Venugopal Taralakshmi, 1994). Treatment based on Indian medicinal plants is becoming increasing and physicians are also looking for alternative treatments because of the side effects of present-day drugs (Gupta, 1994).

Fungi are able to improve resistance against conventional drugs rapidly, prompting the constant need to identify novel antifungal agents. Among the natural sources for therapeutic substances, plants have always played a classic role for such purposes since the dawn of human history. The application of medicinal plants has long been an integral part of both human and veterinary medicine and the practice is still rather common in Asian countries, including China, India, Japan, Pakistan, Sri Lanka and Thailand (Hoareau and Da Silva, 2011).

Traditional medicinal practices, are still playing a major role in the treatment of cattle diseases in developing countries. Farmers rely on traditional medicine because of its affordability and availability (Mohammed Rahmatullah *et al.*, 2010). The possible benefit of plant derived medications constitutes a rewarding area of research, particularly in countries such as India which have a rich biodiversity of

natural plant resources with a high prevalence and variety of infectious diseases (Selvaraju *et al.,* 2011). Sustainable use of medicinal plants in curing the diseases and scientific validation of these herbal preparations are very important so that they can gain equal importance like allopathic drug. The herbal medicines are cost effective and almost have less or no side effects which make them affordable to poor farmers (Dibakar Mishra, 2011).

Pharmacognostical studies

Herbal remedies should be standardized for their active constituents to ensure its quality and clinical reproducibility. Pharmacognosy is concerned with the thorough description and identification of plant drugs in whole state and in powdered form for establishing the herbal drug quality. The quality of secondary metabolites was affected by various environmental factors so, a systematic study of crude drug by pharmacognostical techniques is important for correct identification and quality evaluation (Trease and Evans, 2005).

For the preliminary studies, nine medicinal plants *Allium sativum, Andrographis paniculata*, *Annona squamosa*, *Azardirachta indica*, *Corallocarpus epigaeus*, *Lawsonia inermis*, *Madhuca longifolia*, *Ocimum sanctum* and *Pongamia pinnata* were screened for their antimicrobial property against the fungal pathogens isolated from the skin of diseased animals.

The fungal pathogens identified from the skin scrapings of infected animals were *Aspergillus niger*, *Candida albicans, Curvularia geniculata, Geotrichum candidum, Microsporum gypseum, Penicillium sps, Rhizopus rhizopodoformi, Rhodotorula minuta* and *Trichophyton mentagrophytes.* Bacterial pathogens identified from the skin scrapings were *Staphylococcus sps* and *Streptococcus sps.* The organisms growing on DTM cause the medium to change colour from yellow to red at the time of first visible colony formation. The colour change in the DTM medium is due to the production of alkaline metabolites, which raises pH and

cause the phenol red indicator to change the colour of the medium from yellow to pink-red (Taplin *et al.*, 1969). The fungal pathogens were identified by their morphological characters and the bacterial pathogens were identified by slide or drop catalyse biochemical test.

Among the nine plants screened, *Andrographis paniculata*, *Lawsonia inermis* and *Madhuca longifolia* showed higher inhibitory effect against the isolated pathogens studied and thus these three plants were selected for further studies.

There are number of standardizing techniques applied to evaluate crude drugs. They are loss on drying, ash value, extractive value and fluorescence analysis (Trease and Evans, 2005). Preliminary phytochemical screening was a part of chemical evaluation. The qualitative chemical tests are useful in detection of adulteration. The compounds that are responsible for medicinal property of the drugs are the secondary metabolites (Kokate *et al.*, 2009). In this study, physico-chemical analysis of the selected plant materials was done. Loss on drying was found to be higher in *A.paniculata* than *L.inermis*. This indicated the presence of higher amount of biomass and phytochemical chemical constituents in *A. paniculata*. Total ash content of *M. longifolia* seed was high due to high oil content. The value of acid insoluble ash of *L.inermis* was found to be higher than the other plant materials tested. This is due to the presence of more amount of inorganic compounds (Trease and Evans, 2005). Water soluble ash content was high in *A. paniculata* than the other two plant materials tested.

The extractive value of *A. paniculata* and *L. inermis* was found to be higher in methanol extract. In the previous study it is reported that, extractive value of *A.paniculata* was found to be 12.1% (Abubacker and Vasantha, 2010) whereas in the present study it was 28%. The higher extractive value of *A. paniculata* is due to its higher biomass content and it is reported that methanol is found to be the

suitable solvent to dissolve the phytoconstituents of *A.paniculata* and *L.inermis* (Mamtha, 2011).

In the case of *M. longifolia,* extractive value was high in petroleum ether. This is due to high oil content of seeds (Irfan ali khan and Atiya, 2005). Many substances in plant drugs when illuminated emit light of different wavelength or colour. The selected plant powdered materials treated with different solvents emitted significant variations in colour under normal and UV light. This method was widely used as an identification tool to identify the quality or adulteration in crude drug (Trease and Evans, 2005; Kokate *et al.*, 2009).

The presence of various phytochemical compounds was known to have medicinal importance. In the present study methanol, chloroform and water extracts showed the presence of most of the secondary metabolites like alkaloids, glycoside, flavonids, tannins, phenols, fixed oils, fats, terpenoids, saponins and steroids. Similar findings were reported in methanol extract of *A.paniculata* and *L. inermis* (Divya *et al.*, 2011; Muhammad Ghufran Saeed *et al.*, 2013). *M. longifolia* seed extract contained triterpenoids, steroids, saponins, flavonoids and glycosides (Priyanka yadav 2012). According to earlier report, seed oil contain alkaloid, glycoside, sapogenin, triterpenoids, steroids, flavonoids and other basic acids which are responsible for its medicinal properties (Patel *et al.*, 2012).

All these phytochemical constituents are responsible for curing various ailments in human and animals. Glycosides serve as a defense mechanism against predation by many microbes (De *et al.*, 1999). Steroids are known for their cardiotonic activities, insecticidal and antimicrobial properties (Radha *et al.*, 2011). Phenols and tannins have antioxidant properties and Saponins were used in hypercholesterolemia, hyperglycaemia, antioxidant, anticancer, anti-inflammatory and weight loss (De-lucca *et al.*, 2005).

In the present investigations most of the secondary metabolites are reported in methanol and chloroform extract thus these two extracts were selected for further TLC and HPLC studies. TLC has advantages over other alternative analytical techniques because it is relatively simple, rapid and inexpensive to perform. Methanol extract of *A.paniculata* showed the presence of andrographolide at the Rf value of 0.53 which is slightly deviating from the earlier report where it was detected at the Rf value of 0.58 (Abubacker and Vasantha, 2010). Andrographolide is soluble in methanol. It has a very bitter taste, colourless crystalline in appearance and is called diterpene lactone (Siripong *et al.*, 1992). Other unknown compounds were also deducted in methanol and chloroform extracts which may be among apigenin-7, 4-di-O-methyl ether, andrographolide, flavones, 5-hydroxy-8,2,3-tetramethoxy flavones, monohydroxy trimethyl flavones, andrographin, dihydroxy-di-methoxy flavones, panicelin, d-sisterol, b-esosteroles acids, myricelin and many other compounds reported in *A.paniculata* (Ali *et al.*, 2013).

From the TLC studies of methanolic extract of *L.inermis* showed lawsone at the Rf value of 0.73 along with other two compounds. This is confirmed by the earlier report (Muhammad Ghufran Saeed *et al.*, 2013). It is also reported that, the chief active principle lawsone is soluble in polar solvent methanol (Dhiman anju *et al.*, 2012). Leaves are reported to contain mannitol, tannic acid, mucilage, gallic acid, fats, resins and napthaquinone (Simon *et al*., 1984). The other unknown compounds detected in the methanol and chloroform extracts would be any of these compounds. Methanol and chloroform extract of *M. longifolia* showed five different unknown compounds which would resemble the earlier report that the seed contain a-alanine, aspartic acid, cystine, glycine, isoleucine, leucine, lysine, methionine, proline, serine, Illipene, prosapogenol, beta-sitosterol and sucrose (Chatterjee and Pakrashi, 2000).

HPLC studies were carried out to confirm the existence of andrographolide in *A. paniculta* and lawsone in *L.inermis*. The results showed that andrographolide was detected at the retention time of 2.5 minutes along with 14 different compounds. Similar study was conducted by Meenu Sharma *et al*., (2011) and detected the andrographolide at the retention time of 2.8 minutes. Methanol extract of *L. inermis* confirmed the presence of the lawsone at the retention time of 2.9 minutes. Many other unknown compounds were also detected in HPLC studies.

There are two unknown compounds identified in *M.longifolia* at the retention time of 2.6 and 5.3 minutes. This would be any of the phytoconstituents like saponins, Mi-saponin A, Mi saponins B, fixed oil, oleic acid, myristic acid, a-alanine, aspartic acid, cystine, glycine, isoleucine, leucine, lysine, methionine, proline, serine, Illipene, prosapogenol, beta-sitosterol, sucrose, arginine, glutamic acid, histidine, tryptophan, tyrosine, bassianin, cellulose, oligosaccharides and starch reported in the seed extracts of *M.logifolia* (Priyanka Yadav, 2012).

Secondary metabolites are the essential components of plants. It is important to quantify the chief active component of plants, which plays a major role in curing the ailments. Various factors like age, season, climatic condition, geographical variations and several other physical conditions influence the synthesis of the active phytochemical compounds in the plants which in turn affect their quality and curability.Therefore, efficient methods need to be adopted in identification, extraction, standardization, qualitative and quantitative and pharmacological testings of drugs (Meenu Sharma *et al.*, 2011). Andrographolide is the major active principle of *A. paniculata* responsible for inhibitory activity of pathogens. In this study, andrographolide content was higher in 120 days old plant (just before flowering). Similar result was observed in earlier studies conducted by Renu Parasher *et al.*, 2011. Hence, the leaves can be harvested from 120 days old plants for herbal formulations. With respect to seasonal influence, lawsone content was

found to be higher in fresh leaves than dried leaves collected during the month of july – august. As per the earlier report, lawsone content was found to be higher during october (spring season) and which would be the right season for collecting the plant material for drug preparation (Upadhaya *et al.*, 2010).

Influence of geographical variation was detected by quantitative estimation of Andrographolide and Lawsone content. Both andrographolide and lawsone content were found to be higher in the plants collected from pasumalai hills than from kanjampatty village (Plain). It is suggested that, plants growing under stress condition produce more active principle than plants grown in irrigated land (Upadhyay *et al.*, 2010)

Nanoparticles play a major role in increasing solubility, stability and bioavailability of herbal drugs. Beyond this nano medicines have advantages of protecting from toxins, improving the tissue macrophage distribution, sustained delivery and protection from physical and chemical degradation. Presence of Nano particles can be detected by Scanning Electron Microscope (Neeraj Choudhary and Bhupinder Singh, 2011). SEM analysis of the three selected plant materials confirmed the presence of nano particles. It is reported that, the increased curing activity of herbal drugs are due to the nano particles present in them (Musthaba *et al.*, 2009). Earlier studies revealed that, antimicrobial potent silver nanoparticles were prepared using whole plant aqueous extract of *A. paniculata*. Its effect against bacterial strains such as *B.subtilis*, *E. coli*, *P. aeruginosa*, *P. fluorescence*, *S. aureus*, *S. typhii* and *V.parahaemolyticus* and pathogenic fungi such as *A. flavus* and *A. niger* showed good inhibitory effect on all bacterial species, whereas it showed anti-fungal activity only on *A. niger* and had no effect on *A. flavus* (Rajasekar *et al.*, 2013)

Antioxidant studies

Antioxidants play a major role in protecting biological systems against many diseases. It act as radical scavengers and helps in converting the radicals to less reactive substance thus protecting the body from various diseases (Sulekha Mandal *et al.*, 2009). Since the selected three plants were reported to contain secondary metabolites, they were selected for further antioxidant studies.

The methanol extracts of selected plants showed higher antioxidant property than water extract. As the concentration of the extracts increased the reducing power also increased. The pure andrographolide and Lawsone exhibited highest reducing power than plant extracts. Methanol was found to be the suitable solvent for extracting andrographolide (Mamtha, 2011) and lawsone (Amit *et al.*, 2011). This is the reason for good antioxidant activity of methanol extracts of the tested plant materials. The presence of antioxidants in the plant materials causes the reduction of Fe^{3+} ferricyanide complex to ferrous form (Fe^{2+}) by donating a hydrogen atom and breaking the free radical chain. Thus, exhibiting their antioxidant potency (Savitha and Rathnavijaya, 2011).

***In vitro* studies**

Determination of antimicrobial property of medicinal plants is essential to identify the existence antimicrobial components. The antimicrobial property of the plants is mainly due to the presence of secondary metabolites such as alkaloids, flavonoids, tannins, phenols, saponins and several other aromatic compounds which serve as defense mechanism against many microorganisms, insects and other herbivores (Bonjar, *et al.*, 2004).

The antimicrobial studies of the present work revealed that, *Andrographis paniculata, Lawsonia inermis* and *Madhuca longifolia* when used as single drug inhibited the isolated fungal and bacterial pathogens. Among the three plants tested *A.paniculata* exhibited good inhibitory effect than the other two plants. It is stated that, the methanolic extract of *A. paniculata* had broad spectrum antimicrobial

property (Divya *et al.*, 2011). It is also reported that, *A. paniculata* exhibited good antibacterial activity against the skin infection causing bacterial strains (Sule *et al.*, 2010). The antimicrobial property of the plants is mainly due to the presence of secondary metabolites like alkaloids, glycosides, flavonoids, tannins, phenols, saponins, terpenoids and quinines are reported in the preliminary phytochemical studies.

Beyond this, andrographolide and lawsone are also reported in methanol extract of these plants which posses high antioxidant property is responsible for antifungal activity. The plants when used as single exhibited good antimicrobial activity. So these plants are tried in combinations of two and three in 1:1 and 1:1:1 ratio respectively. Plants in combinations of two (1:1) exhibited higher activity than the plant extracts used as single. Among the combinations tried combination of *L.inermis* and *M.longifolia* was found to be good. This is due to the combination of the active principle Lawsone in *L.inermis* and various important phytochemicals in *M.longifolia*.

When combination of three plants in 1:1:1 ratio tried it was found better than single as well as in combination of two plants. This is due to the combined or synergistic effect of the poly herbal drugs. Synergy broadly means "working together". It is insisted that better results obtained in combination of many plants is used rather than using isolated compounds (Trease and Evans, 2005; Irfan ali khan and Atiya, 2005).

The inhibitory effect of methanol and water extracts was equally good in combination of three plants. It was observed that the extractive value of methanol was higher in *A. paniculata* and *L. inermis* and also reported that the preliminary phytochemical analysis gave positive result to the presence of all secondary metabolites. This is the reason for the good inhibitory effect of methanol.

Since the water is inexpensive and has no side effects water extract was selected for further studies. MIC studies were carried out in the plant extracts to determine the minimum required concentration to kill the pathogens. Inhibitory effect was increased as the concentration increased. Owing to the MIC studies of the selected plants and their combinations, all the three plants required minimum dose for inhibiting the organisms. This concentration varied with the pathogens yet, the combination of three plant extracts required very minimum dosage for inhibiting the organism compared to the single drug and combination of two plant extracts.

***In vivo* studies**

From the *in vitro* studies, the three plant extracts in combination (1:1:1) showed best result against all the fungal pathogens tested and hence this combination was selected for *in vivo* studies. This *in vivo* study was conducted to test the efficacy of drug under field condition and also to standardize the drug dosage for ointment preparation. The infected animals treated with this plant extracts cured faster at the concentration of 400-500 µg than the allopathic drugs used. The curability of plant extracts are mainly due to the combined action of the three plant extracts.

Preparation of herbal ointments

Ointments are used as skin conditioners and as a mean of treating skin infections, they are mostly simple to make with the base ingredients being readily available. Ointments are fatty preparations of a softer consistency than waxes. Petroleum jelly, soft paraffin wax and bees wax are some common bases used in ointment (Ariamuthu Saraswathy, 1994). For acceptance of any herbal drugs, it should be prepared following the standard “Pharmacopeia”. The need for standardization includes study from germination of the plant to clinical application.Standardization methodology includes, collection or procurement of

raw drugs, systematic identification, method of purification or detoxication, methods of manufacture, finished produce standardization, period of preparation, preservation and shelf life (Siddha parmacoepia, 2005).

The delivery of drug through the skin has long been a promising concept because of the easy access, large surface area, vast exposure to the circulatory and lymphatic networks and non invasive nature of treatment. After standardization of the herbal formulation clinical trials may be conducted to prove the efficacy of the prepared formulations (Daniel and Knie, 2007).

In the present study, the herbal ointments are prepared based on the above mentioned prescribed standard methods of Siddha pharmacopeia (2005). The ointment preparation was done following the traditional method (prescribed in Gunapadam) and industrial method (modified method of Viyoch *et al.*, 2003). The ingredients used for the preparation was same but the method of preparation varied in traditional and industrial method of ointment preparation. The two methods were preferred to check whether there is any difference in their curing property when methodology is altered.

Evaluation of herbal ointments

Since the three plant extracts in combination showed good result in curing the disease, this combination was used in the preparation of herbal formulations. It is essential to evaluate various parameters to meet the prescribed quality of drugs for acceptance in the market (Siddha pharmacopeia, 2005). The evaluation parameters like pH, viscocity, spreadability, homogeneity, stability and primary skin irritation test were important to test the quality of ointments before use. The pH of the ointments were found to be in range of 6.8 and 6.9 which is reported to be good for skin (Pattanayak *et al.*, 2011). The viscosity of ointments in the

optimal range indicated that the ointments are easily spreadable by small amounts of smear (Beena *et al.,* 2010).

Spreadability helps in uniform application of ointments on the skin. A good ointment takes less time to spread and will have high spreadabiltiy. In the present investigation spreadability of the prepared ointments were 10.6 and 10.4 for traditional and industrial ointments respectively. It is supported by the earlier report that, spreadability of ideal formulation was found to be 10.5 (Deborah *et al.,* 2011). Hence, the ointments have optimal spreadability.

Stability studies were also carried out to find out the shelf life of ointments in different temperature conditions. All the parameters evaluated were found to be ideal till 90 days as mentioned in Siddha pharmacopeia (2005).

Primary skin irritation test revealed that there is no formation of erythema or edema on animals. Similar result was obtained in a study conducted calf with a poly herbal formulation by Mandeep Singh (2011). The ingredients in the ointment were non allergic and non irritant for animals skin hence the ointments did not develop any erythema or edema. Since the ointments were non irritant they are safe to use for treatment.

Heavy metal studies

Plant species greatly differ in their ability to uptake, accumulate and tolerate heavy metals (Kursat Korkmaz *et al.,* 2010). WHO recommends that medicinal plants which form raw materials for the finished products may be checked for the presence of heavy metals (WHO, 1998). Heavy metal ions in contaminated soils may easily enter the human and animal food chain through crop plants (Peris *et al.,* 2007). Accumulation of heavy metal causes serious health problems like high blood pressure, hyperactivity of the nervous system and kidney diseases (Iqbal Hussain and Lajberkhan, 2010). In this study, the plant materials along with the soil from the habitat and the ointments were analyzed for the presence of heavy

metals like lead, chromium, nickel and cadmium. The lead and chromium content was found to be higher in *L.inemis* but they were under the permissible level. The accumulation of nickel and cadmium contents were higher in *M. longofolia* seeds and the soil sample collected from their habitat. This indicated that the accumulation of heavy metals in seeds due to the contamination of their habitat. All the elements analysed in the selected materials including ointment were found within the permissible level set by WHO. This results confirmed that the ointment are safe to use.

Clinical studies

Clinical trials were conducted with the prepared ointments on infected domestic animals such as cows, dogs, goat, sheep, horse and ox under field condition. Animals from different geographical region and different breeds were randomly selected to check the curability of ointments under varied climatic conditions and in various breeds. Infection on animals varied from mild to severe. The efficacy of ointments were determined by the number of days taken for complete recovery of animals. Animal recovery rate was appreciable with herbal ointments than standard allopathic drugs povidone iodine and curabless used. Herbal formulations took minimum number of days to cure the animals. But the allopathic drug took more days for recovery. Since the allopathic drugs took more days for recovery the ointment required for treatment will also be more. This leads to accumulation of chemicals on the skin of animals which will cause side effects (Aiello and Mays, 1998). The herbal ointments cured the infection faster and the ointment requirement will be lesser. Since the herbal ointments are made of herbal ingredients, accumulation of the phytochemicals on the skin will not cause any serious side effect. The herbal ointments are also cheaper. Beyond this, skin texture and colour were also found to be improved. Reoccurrence of the disease in the animals treated with herbal ointments was not noticed which is an important

criteria to be noted.

This efficacy of the herbal ointments was mainly due to the presence of active phytoconstituents in the plants. The collection of plants in proper time, season and geographical region were also other factors affecting the efficacy of the drug. Presence of nanoparticles is also an influencing factor. The efficacy of the drug is due to the combined or synergistic effect and action of phytochemicals of the selected plants. All these factors together contribute to the curing ability of the ointments. The ointment is also highly economical because the plants used for the preparation are common and available everywhere. The oil bases paraffin and bee wax are also affordable. *M.longifolia* oil is also available in market at reasonable price. Considering all these, it is evident that the ointment preparation cost is economical. It is important to be noted that, the ointments are devoid of heavy metal contamination and hence, no side effects will be occured.

Thus it is concluded that the herbal ointments developed are economical, safe and has good curability with no side effects.Hence, it can be recommended for treatment of skin disease on domestic animals.

The ointment is filed for patent. Patent number 5029/CHE/2012 (Copy of publication of patent in The official Journal of Patents attached-Annexure).