Evaluation of Topical Antifungal Activity of *Gomphocarpus purpurascens* A. Rich Leaves and Stems Extracts Against Epizootic Lymphangitis

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**Abstract:** In Ethiopia, high curative evidence was noticed in wound management and wart treatment, where people using ‘tifirgina (local name)’ as a medicament. The extract was filtered by using Whatman filter paper (540 hardened ash less circles, 110mm thickness CAT No. 1540 110). The solvents from the crude extract were removed by using a rotary vacuum evaporator with the water bath temperature of 50°C. Clinical signs were cutaneous lesions in the neck region and forelimbs. The classical epizootic lymphangitis form was seen in these cases. The three male horse’s were treated by topical application of *Gomphocarpus purpurascens* A. Rich leaves and stems extracts. The treatment regimen was assessed clinically and proved to be effective. Some veterinary and public health aspects of the disease are discussed.

**Key words:** *Gomphocarpus purpurascens* • Extract • Epizootic lymphangitis • Horse

**INTRODUCTION**

Epizootic lymphangitis (EZL) is a contagious relatively common infectious disease of horses and other equids caused by the dimorphic fungus, *Histoplasma capsulatum variety farcinosum* [1, 2]. It is a debilitating disease. Most cases of EZL are reported from horses (90%) and the remainder from mules and donkeys. EZL infection can occur in camels, cattle and dogs [2]. EZL infection in humans has also been reported [3, 4]. Cattle are more resistant than equids [3].

Epizootic lymphangitis is a chronic granulomatous disease of the skin, lymph vessels and lymph nodes of the limbs, chest and neck of Equidae. It is clinically characterized by a spreading suppurative inflammation of cutaneous lymphatic vessels, lymph nodes and adjacent skin [5, 6]. Epizootic lymphangitis is a common infectious disease of horses in Ethiopia [7]. It is a significant concern in the country, where the prevalence in carthorses is nearly 19% and economic losses from this disease are high [5, 7]. EZL is particularly prevalent in the study area [7]: it is 24.9% for instance in Woliso [8]. It causes significant impact on the carthorse owners, that they considered it as the first and as a major disease of horses in Ethiopia. EZL causes an average loss of 129 USD incurred per annum per owner as a result of death of affected horses and an average 2.5 USD decline from the net profit per day due to reduced working performance of affected horses [9].

The causative agent of EZL is *Histoplasma capsulatum variety farcinosum* (HCF). It is a thermally dimorphic fungal soil saprophyte. The mycelial form is present in soil, while the yeast form is usually found in lesions [10]. HCF is highly resistant to the effects of physical and chemical agents. It may survive for up to ten weeks in non-sterile water at 26°C [11-13].

**The Aim(s) of Study:** The main aim of the study was to conduct whether the *Gomphocarpus purpurascens* has antifungal activity or not.

**MATERIALS AND METHODS**

**Plant Selection and Identification:** In this study, a plant was selected from primary data. Plant species of *Gomphocarpus purpurascens* A. Rich (Family Asclepiadaceae – Milkweed family) showing toxicity properties was chosen. Since the peoples have used this plant as a medicament agent for numerous ailments like wound treatment as well as for wart treatment. In Ethiopia, high curative evidence was noticed in wound management and wart treatment, where people using ‘tifirgina (local name)’ as a...
medicament. The collected voucher specimen has been identified, pressed, numbered, dried and deposited in the Gondar University Regional Herbarium, Ethiopia.

**Preparation of Crude Extract from the Fresh Leaves and Stems:** The fresh leaves and stems of tifirgina were collected from the outskirts of Gondar town, Amhara Region, Ethiopia and brought to Environmental Biology laboratories of Gondar University. The leaves and stems together were shredded into pieces subsequently 200g of the shredded leaves and stems were taken and crushed to obtain paste by using a mortal pistil grinder. Then, the paste was dissolved in 400ml distilled water. The mixed solution was kept for 24 h and then the solution was filtered with cheese cloth and filter paper [14]. The filtered fresh leaves and stems (crude) extract was stored in the refrigerator at 4°C for later use. Then 200ml of the extract was manually applied on live animals which were diseased by epizootic lymphangitis.

**Preparation of Acetone, Methanol and Petroleum Extracts from the Dried Leaves and Stems:** Tifirgina leaves and stems were shade dried at room temperature for 30 days and the dried leaves and stems were powdered by using electrical blender. The 300g tifirgina powder was kept in three conical flasks with 1000ml capacity and 600ml of acetone, methanol and petroleum (solvent) were poured into each flask. Mouths of flasks were covered with aluminum foil and placed in a water bath for 72 h for continuous agitation for thorough mixing and complete elucidation of bio-active molecules to dissolve in the solvent [15]. Then the extract was filtered by using Whatman filter paper (540 hardened ashless circles, 110mm thickness CAT No. 1540 110). The solvents from the crude extract were removed by using a rotary vacuum evaporator with the water bath temperature of 50°C. Finally, the residues of dried leaves and stems were collected in a vial (also known as a phial or flacon) and stored in refrigerator at 4°C for subsequent usage.

**Case History:** Three North Gondarian, Ethiopia male horses (2.5 and 3.5 – years –old) with skin infection were encountered in Gondar University veterinary clinic. Clinical examination revealed lesions of 2 years old on the neck region and 5 and 3.5 – years –old on the forelimbs. In the affected areas there were cutaneous lesions. The infection was not limited to the skin and had visible involvement of the lymphatic vessels. Those male horses had good general body condition, normal body temperature and normal respiration and heart rates.

**Treatment:** Affected parts of horses were cleaned and the extract was topically applied on for five days. Each horses were used around 67ml of the extract for five days. This was done without hair shaving.

**RESULTS**

The treatment regimen was well tolerated with no apparent local reactions or side effects. The lesions started to improve after few days and were unapparent after three weeks. Clinically, no recrudescence was observed at the end of the observation period. Figures 1-16 show that the fresh leaves and stems distilled water extracts of *Gomphocarpus purpurascens* A. Rich have excellent antifungal properties against epizootic lymphangitis.

Fig. 1: Skin lesion in 2 years old horse showing in the neck region and treatment day one.
Fig. 2: Skin lesion in 2 years old horse showing in the neck region and treatment day five.

Fig. 3: Skin lesion in 2 years old horse showing in the neck region and treatment day ten.

Fig. 4: Skin lesion in 2 years old horse showing in the neck region and treatment day fifteen.

Fig. 5: Skin lesion in 2 years old horse showing in the neck region and treatment day twenty.
Fig. 6: Skin lesion in 5 years old horse showing in the forelimb region and treatment day one.

Fig. 7: Skin lesion in 5 years old horse showing in the forelimb region and treatment day five.

Fig. 8: Skin lesion in 5 years old horse showing in the forelimb region and treatment day ten.

Fig. 9: Skin lesion in 5 years old horse showing in the forelimb region and treatment day fifteen.
Fig. 10: Skin lesion in 5 years old horse showing in the forelimb region and treatment day twenty.

Fig. 11: Skin lesion in 5 years old horse showing in the forelimb region and treatment day twenty five.

Fig. 12: Skin lesion in 3.5 years old horse showing in the forelimb region and treatment day one.

Fig. 13: Skin lesion in 3.5 years old horse showing in the forelimb region and treatment day five.
Fig. 14: Skin lesion in 3.5 years old horse showing in the forelimb region and treatment day ten.

Fig. 15: Skin lesion in 3.5 years old horse showing in the forelimb region and treatment day fifteen.

Fig. 16: Skin lesion in 3.5 years old horse showing in the forelimb region and treatment day twenty.

DISCUSSION

In this cases, despite the fact that systemic infection was not diagnosed, the body failed to get rid of the infective agent before administration of medicinal treatment. To the best of my knowledge, there was no record of spontaneous recovery from this disease in the literature. The application of the extract was effective and safe. This treatment regimen was chosen as the infection was confined to the skin and cutaneous tissues.

These reports indicate that the extract of *Gomphocarpus purpurascens* A. Rich can be useful as a safe and low-cost possible alternative to costlier synthetic fungicidal in the epizootic lymphangitis control attributable to its bio-degradable nature. However, further research is warranted to explore the efficacy and safety of Tifirgina-based fungicide towards the human beings, livestocks and environment to formulate and market it as a potential fungicidal agent to control fungus of equine health importance, especially epizootic lymphangitis in the future. Interestingly, tifirginais one of the endemic plants and widely distributed across Ethiopia and the rest of the Africa. It provides an ideal opportunity for us to generate the income among the rural poor by cultivation as well as
to develop and deploy risk reduced (ecological) fungicidal from the tifirgina plants. We hope it could contribute to substantial alleviation of poverty in this nation in the future.

The disadvantages that may curtail the use of topical application are the need to handle cases separately and personnel exposure to infection.

**CONCLUSIONS**

The present study results apparently exhibit that the candidate plant tifirgina has remarkable antifungal activity against epizootic lymphangitis. Since, epizootic lymphangitis has developed resistance against most of the conventional fungicidal. In this context tifirgina could play a pivotal role as a potent epizootic lymphangitis control agent in the near future. Withal, this communication warrants further investigations to elucidate the responsible bio-active principles, mode of action and safety. Besides adequate measures have to be made by bringing a multi disciplinary act, with a range of pertinent stakeholders in order to formulate and commercialize the tifirgina based fungicidal in the future.

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**REFERENCES**