

Euphorbia Sanguinea stem sap as antifungal agent against Onychomycosis

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Abstract

The work focuses of the antifungal activity of *Euphorbia sanguinea* stem sap against onychomycosis causing fungi as well as its phytochemical composition and antibacterial activity. The work shows that the sap has no activity against *Escherichia coli*, *Staphylococcus albus*, *Staphylococcus aureus*, *Proteus mirabilis*, *Samonela thyphi* and *Klebsiella pneumoniae*. It contains flavonoids and saponins but lacks tannins, alkaloids, terpenoids and anthraquinones. The sap shows a strong antifungal activity when compared with the conventional antifungal agents in the market. It has activity against certain fungi responsible for onychomycosis. It is therefore a suitable natural substitute for the conventional antionychomycotic agents.

Keywords: *Euphorbia sanguinea*, antifungal, phytochemicals, physiochemical properties, antibacterial activity, antipsychotic agent

1. Introduction

Onychomycosis or *Tinea unguium* is a nail fungal infection. This is the most common disease of the nail in man. They usually manifest in discolouring and thickening of the nail and crumbling edges. The prevalence of onychomycosis is about 6-8% in adult population (Sigurgeirsson and Baran, 2013) [12]. The most common causative agent is *Trichophyton rubrum* (Kaur *et al*, 2008) [9]. Others include *T. interdigitale*, *T. violaceum*, *T. tonsurans*, *T. soudeneses* and *Epidermophyton flexuosum*.

Nail fungal infection can be painful and may cause permanent damage to the nail. They pose more serious health hazard for people suffering from diabetes or with weakened immune system. Treatment of onychomycosis is challenging because it is embedded within the nail and is difficult to reach. Full removal of the symptoms with the drugs, currently in use in orthodox medicine, is slow and may take a year or more. It is against this background that the search for drugs for control of onychomycosis is directed to the plant kingdom. *Euphorbia sanguinea* stem sap is locally used against onychomycosis by a certain alternative medical practitioner. Topical application cures onychomycosis in few days. This plant species has been botanically identified (Bruyns, 2012) [13]. It appears, to the best of our knowledge, that there are no reports on its chemical composition and antimicrobial activity. The present work is therefore focused on the phytochemical composition and antimicrobial activity of *Euphorbia sanguinea* stem sap.

2. Materials and Methods

Fresh sap of *Euphorbia sanguinea* stem was collected in July from plants growing in Calabar, Cross River State, Nigeria. The plant was authenticated by Frank Apeoye, head, Herbarium Unit, Botany Department, University of Calabar. The sap was tested for the presence of alkaloids and saponins using the methods of Sofowora (1998), tannins and anthraquinones by the methods of Trease and Evans (1978) [14], flavonoids by the methods Guilei (1982) and terpenoids by Liebermann test.

Moisture content was determined by drying at 100 °C in the

oven to a content weight. Protein was determined by Kjeldahl method. Ash content, acid insoluble matter, and hot and cold water insoluble matter were determined by standard methods (Pomeranz and Meloan, 2004) [11].

Antimicrobial activity of the fresh stem sap was conducted on the following pathogens: *Escherichia coli*, *Staphylococcus albus*, *Staphylococcus aureus*, *Proteus mirabilis*, *Salmonella thyphi* and *Klebsiella pneumoniae*.

Susceptibility test of the fresh sap and some conventional synthetic antifungal agents on the cultured crude nail fungi was conducted using the method of Bauer *et al* (1996) [2]. The antifungal agents used are fluconazole, itraconazole, posaconazole, voriconazole, amphotericin B, flucytosine, caspofugin and griseofluvin.

The nail samples each of about 1g was collected from 150 patients with clinically suspected onychomycosis at the pathology and microbiology laboratory of the University of Calabar Teaching hospital. The nail scrapings were subjected to potassium hydroxide mount for direct microscopic and histopathological examinations with periodic acid-Schiff (HP/PAS) for microbial detection. A culture test for nail scrapings were conducted on sabouraud's dextrose agar plate. The following fungi were isolated: *Candida albicans*, *Candida glabrata*, *Aspergillus flavus* and *Trichophyton rubrum*. Antifungal activity of the fresh sap was also conducted on the isolated fungi.

3. Results and Discussion

Table 1: Phytochemical constituents of *Euphorbia sanguinea* stem sap

Constituent	Result
Tannins	-
Alkaloids	-
Flavonoids	+
Saponins	+
Terpenoids	-
Anthraquinones	-

Key: += Present; - = absent

Table 2: Physicochemical properties of *Euphorbia sanguinea* stem sap

Parameter	Value
Moisture % w/w	22.9
Ash % w/w	0.1
Acid insoluble matter % w/w	10.0
Hot water insoluble matter % w/w	5.7
Cold water insoluble matter % w/w	4.6
Nitrogen % w/w	2.8
Viscosity 100gdm ⁻³ pas	1.27
Viscosity 150gdm ⁻³ pas	1.53

Table 1 shows the phytochemical constituents of *Euphorbia sanguinea* stem sap. It contains flavonoids and saponins but lacks tannins, alkaloids, terpenoids and anthraquinones. Table 2 shows the physicochemical properties of *Euphorbia sanguinea* sap. It has low moisture content of 22.9%. It has very low ash content of 0.1% indicating that it is deficient in minerals. The nitrogen content is relatively low and it is relatively viscous. The sap showed no antibacterial activity against all the test organisms: *Escherichia coli*, *Staphylococcus albus*, *Staphylococcus aureus*, *Proteus mirabilis*, *salmonella thyphi* and *klebsiella pneumonia*.

Table 3: Minimum inhibitory concentration for antifungal susceptibility on the crude fungal extract.

Antifungal	MIC mgcm ⁻³
Posqconazole	0.016
Voriconazole	0.03
Itraconazole	0.125
Caspofugin	0.125
<i>Euphorbia sanguinea</i> sap	0.125
Amphotericin B	0.25
Flucytosine	2.0
Griseo fluvin	2.50

Table 4: Activity of *Euphorbia sanguinea* sap against insolated fungi from infected nails

Isolate	Activity
<i>Candida albicans</i>	++
<i>Candida glabrata</i>	++
<i>Aspergillus flavus</i>	+
<i>Trichophyton rubrum</i>	+

Key: ++=very strong activity; += moderate activity

Table 3: shows minimum inhibitory concentration for *Euphorbia sanguinea* sap and some other synthetic drugs used in orthodox medicine for the treatment of onychomycosis. The sap showed a high antifungal activity when compared with the available synthetic antifungal drugs in the market for the treatment of onychomycosis. This justifies the local use of *Euphorbia sanguinea* sap for the treatment of onychomycosis. Table 4: Shows the antifungal effect of *Euphorbia sanguinea* stem sap against the isolated nail fungi. It has a very strong antifungal activity against *Candida albicans* and *Candida rubrum*. Flavonoids are known to have strong antifungal activities (Kanwal *et al*, 2010, Alle *et al*, 2012, Clara *et al*, 2014) [8, 1, 4]. Saponins are also known to exhibit antifungal activities (Tsuzaki *et al*, 2007. Njateng *et al*, 2015) [15, 10]. It is therefore conceivable that the flavonoid and saponin contents of the sap are responsible for its effective use against nail

fungi. Isolation of the active principles of the sap may open a new door for discovery of new drugs against nail fungi.

4. References

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