

Antimicrobial activity and phytochemical investigation of crude extracts of the fruits of *Solanum incanum* (Solanaceae) and *Dovyalis abyssinica* (Flacourtiaceae)

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ABSTRACT

The aim of this study was to investigate the *in-vitro* antimicrobial activity and to test the presence of phytochemicals in methanolic crude extracts of the fruits of *Dovyalis abyssinica* and *Solanum incanum*. The antibacterial and antifungal activities of methanol extracts of *Solanum incanum* and *Dovyalis abyssinica*, respectively, were carried out by agar diffusion assay technique against one bacterial strain, *Staphylococcus*; and one fungal strain, *Trichophyton rubrum*. Crude extracts of both plants showed activity against both the fungus and the bacterial test microorganisms. The methanolic crude extracts of both plants revealed the presence of phytochemicals such as alkaloids, flavonoids, tannins, phenols, saponins and in one case proteins. This study provides some scientific basis upon which these two traditional medicinal plants have found usage in the treatment of a variety of ailments.

KEYWORDS: *Staphylococcus*, thnobotanical, *Trichophyton rubrum*, *Solanum incanum* and *Dovyalis abyssinica*

INTRODUCTION

Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube *et al.*, 2008). Since time immemorial, different parts of medicinal herbs have been used to cure specific ailments in Kenya (Karinge, 2006). The medicinal value of the herbal secondary metabolites is due to the presence of chemical substances that produce a definite physiological action on the human body (Maobe *et al.*, 2013). Plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of diseases (Shakti S.S and Selvanayagam M., 2013). Many of these natural products have been shown to present interesting biological and pharmacological activities and are used as chemotherapeutic agents or serve as the starting point in the development of modern medicines (Abubakar *et al.*, 2010).

Solanum incanum is an herbaceous or soft wooded shrub, 0.5-3m in height. It is a very variable species which may be armed on most parts including the leaves or may occasionally be unarmed. Stems and undersides of leaves may be densely covered in a whitish felt of stellate hairs. Fruit is spherical, green, often striped with white, turning yellow to orange-brown when ripe.

Dovyalis abyssinica (A. Rich) Warb is a spiny evergreen shrub or tree, up to 5m height, with a rounded crown. The bark is ash grey, almost always supporting lichens. Branches armed with stout spines, up to 1½ cm long. The branchlets are covered with numerous dotted pores (lenticels). Leaves are oval to obovate, up to 5-7 cm long and 3 cm wide with a rounded tip, edges unevenly rounded, shiny, dark green, with reddish stalks and veins. Flowers are unisexual, yellow-green or greenish without petals, 5-7 mm long. Female flowers are single or found in 2-3 flowered fascicles. Male flowers present in clusters, with 40-60 stamens.

The fruit is edible (eaten raw), but very acidic. It is used for making jam and is added to porridge as a flavouring. Roots and stem are good for making soup. The roots also have medical properties with alleged effect on gonorrhoea, bilharzias, stomach-ache and fever. The leaves provide fodder for livestock, primarily goats and sheep. Flowers attract bees and the plant is often used as a live fence. Even though some studies might have been done on these plants or from the same families, the qualitative analysis of these plants from the study region may not have been evaluated leading to the current study. The study aims at providing a scientific basis for the traditional claims of this ethno-medicinal plants and in evaluating the actual value of folklore remedies.

2.0 MATERIALS AND METHODS

2.1 Plant collection and identification

Fresh samples of *Dovyalis abyssinica* and *Solanum incanum* were collected from natural undisturbed areas around University of Kabianga locality (around a radius of 3km from the university) because of their availability following previous field survey. Twigs containing fruits were cut from the stem of a young *Solanum incanum* plant and *Dovyalis abyssinica* of about 6 month maturity. Afterwards, the plant materials were put in a polythene paper bag and then transferred to University of Kabianga laboratory for verification. The plant materials were further identified by microscopic method under the aid of a botanist from School of natural resource, University of Kabianga.

2.2 Preparation of Plant Samples

The collected plant samples were washed thoroughly under running tap water to get rid of dirt and any other soluble

impurities rinsed with deionized water and were air dried under shade for about three weeks. The dried plants were ground by use of a clean and dry electric blender and the powdered materials were then stored in small air-tight plastic sample containers with proper labeling.

2.3 Preparation of crude extracts

Preparation of crude extracts was undertaken using the extraction procedure of maceration. The powdered materials weighing about 200g were extracted using analytical grade solvent. It was extracted with the methanol solvent for a period of 72 hours with constant agitation using magnetic stirrer and filtered using Whatman no.1 filter paper. About 300ml of extracts was obtained which was concentrated to 50ml using rotary evaporator and was stored in fridge awaiting further analysis.

2.4 Preparation of standard bacterial and fungal culture

Standard nutrient agar was prepared by dissolving 18grams of the agar into 500ml of distilled water. This was brought to boiling to completely dissolve the agar. The solution was then poured into Petri dishes and left to set for 10 minutes. By use of an inoculating loop, several streaks of ear wax bacteria were made on the solid nutrient agar. This was incubated for 24 hours. The same was done for fungi, that is, the fungus causing athletes' foot using potato dextrose agar (PDA) instead of nutrient agar. This was also incubated for 24 hours.

2.5 Phytochemical analysis

Qualitative phytochemical analysis of the crude extracts of *Solanum incanum* and *Dovyalis abyssinica* for the tests of phytochemicals as alkaloids, saponins, flavonoids, proteins and tannins were done using standard methods as per the methods of Sofowora (1984) and Harbone (1984).

2.5.1 Test for alkaloids

Approximately 2 ml of filtrates in a clean test tube were treated with 2-3 drops of Wagner's reagent (iodine in potassium iodide) and the test tube immersed in a boiling water bath for about five minutes. The sample was then cooled and observed for formation of a reddish brown precipitation or turbidity which indicates the presence of alkaloids (Harbone, 1973).

2.5.2 Test for flavonoids

This was done using Alkaline Reagent Test where 2 ml of filtrates were treated with 2-3 drops of Sodium Hydroxide solution at room temperature. The sample was shaken and observed for formation of intense yellow color which becomes colorless on addition of dilute acid, indicating presence of flavonoids.

2.5.3 Test for Tannins and phenols

To 0.5 ml of extract solution, 1 ml of distilled water was added and heated in a water bath for about five minutes then cooled.

Afterwards, 1 to 2 drops of ferric chloride solution was added to the sample, this was shaken and observed for blue or green black precipitation which indicates presence of tannins and phenols.

2.5.4 Test for saponins

Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. The sample was, then observed for frothing persistence or formation of 1cm layer of foam which indicates the presence of saponins. If no frothing occurs, that indicates no saponins.

2.5.5 Test for protein and amino acids

To the plant extracts about 2 ml, 0.25% w/v ninhydrin reagent was added and boiled for a few minutes. This was left to cool and then observed for the formation of blue color which indicates the presence of amino acids.

2.5.6 Antimicrobial and antifungal activity

The antimicrobial and antifungal activity of the prepared extracts was determined by using paper agar diffusion method. The cultured bacteria were inoculated from their Petri dishes and mixed with 30 ml sterile nutrient agar thoroughly. The inoculated nutrient agar was poured into clean sterile Petri dishes, this was left to set then small round pieces (about 0.5 cm wide) of filter paper initially soaked in the extracts were placed on the set inoculated nutrient agar.

The plates were incubated at 36°C for 48 hours. Two replicates were also performed for each extract against each of the test organism. Simultaneously addition of respective solvents instead of extracts was carried out as controls. The same procedure was done for the fungi but the agar used was (PDA).

3.0 RESULTS AND DISCUSSION

Antibacterial and antifungal activity was positive for the methanolic extracts of the two plants. The highest zone of inhibition was shown by *Solanum incanum* against *Staphylococcus* and *Trichophyton rubrum*.

This assay was done based on the indigenous knowledge that fruit extract of the two medicinal plants can be applied on open wounds and in the case of *Solanum incanum* as ear lotions.

The results supports the ethno-botanical usage of the plant in managing those disorders. The antimicrobial activity has been summarized in Table1 below.

The preliminary phytochemical screening for the presence of alkaloids, flavonoids, tannins, phenols, saponins and proteins was carried out on the aforesaid extracts and the results revealed that the above mentioned phytochemicals were all present in *Solanum incanum* extracts whereas in *Dovyalis abyssinica*, all were present except for proteins. Observations for the phytochemical screening of *Solanum incanum* and *Dovyalis abyssinica* were tabulated in Table 2 and Table 3, respectively.

Table 1. Antimicrobial activity of *Solanum incanum* and *Dovyalis abyssinica* methanolic crude extracts

Test microorganism	Zone of inhibition (mm)	
	<i>Dovyalis abyssinica</i>	<i>Solanum incanum</i>
Staphylococcus	7.2±0.1	10.1±0.3
Trichophyton rubrum	8.6±0.2	10.4±0.1

Table 2: Phytochemical screening of *Solanum incanum*

Phytochemicals	Test	Observation	Inference
Alkaloids	Wagner's test	Formation of reddish brown precipitate	Presence of alkaloids
Flavonoids	Alkaline test	Formation of intense yellow color	Flavonoids present
Tannins	Ferric chloride test	Formation of a dark green solution	Tannins present
Saponins	Froth test	Frothing persistence	Saponins present
Proteins	Ninhydrin test	Formation of blue color	Proteins present
Phenols	Ferric chloride test	Formation of a bluish black solution	Phenols present

Table 3: Phytochemical screening of *Dovyalis abyssinica*

Phytochemical	Test	Observation	Inference
Alkaloids	Wagner's test	Reddish brown precipitate	Alkaloids present
Flavonoids	Alkaline test	Formation of intense yellow color	Flavonoids present
Tannins	Ferric chloride test	Formation of a dark green solution	Tannins present
Saponins	Froth test	Frothing persistence	Saponins present
Proteins	Ninhydrin test	Formation of yellow color	No proteins
Phenols	Ferric chloride test	Formation of deep blue color	Phenols present

The fruits of *Solanum incanum* Linnaeus, also known as thorn apple, bitter apple, sodom apple, are extensively used by the Kipsigis sub-tribe from Kabiana division, Kericho County, Kenya for the treatment of cutaneous mycotic infections and other pathological conditions. The therapeutic activity of the berries from other studies outside this region had attributed to their content of solanine and related glycoalkaloids, which are saponins and cytostatic poisons. Its extracts are also used in traditional Indian medicine for the treatment of various ailments like rheumatism, piles, fever, skin diseases and snake bites (Pavithra PS *et al.*, 2010).

The presence of **tannins** in both the plants showed that the plants could be used as purgatives. They are also used in the

treatment of cough, asthma and hay fever (Egwaikhide *et al.*, 2007). Tannins also have the ability to react with proteins to form stable water insoluble components. Since bacteria cell walls are made of proteins, tannins are seen as active detoxifying agents by precipitating the protein components of bacteria hence inhibiting their growth. In this study, the presence of tannins might have accounted for the sharp taste of *Solanum incanum* and have been reported to hasten healing of wounds and inflamed mucous membrane.

Alkaloids are well known Phytochemicals especially for their broad pharmacological activities including antibacterial and antifungal. The biological function of alkaloids and their derivatives are very important and are used in analgesic,

antispasmodic and bactericidal activities (Iqbal *et al.*,2011). Morphine, quinine, ephedrine, nicotine and strychnine are the additional types of alkaloids. In these types, morphine and codeine are narcotic analgesics as well as anti-tussive agent (Stary, 1998).

Saponins are naturally occurring surface active glycosides which have soapy characteristics (Iqbal *et al.*, 2011). The presence of saponins justifies why the extracts from these plants are used in wound healing and bleeding treatment. In addition, Saponins have been reported to possess properties of precipitating and coagulating red blood cells and they also have cholesterol binding properties, formation of foams in aqueous solutions and hemolytic activity (Sodipo *et al.*, 2000). Due to the potential activity of saponins as antibacterial agents, their presence serves as the indicator towards possible antibacterial activity (Oakenful, 1986).

Flavonoids are water soluble phytochemical and an important plant phenolic. They show antioxidant activities and they have the property of preventing oxidative cell damage and carcinogenesis. They have anti cancer and anti inflammatory activities, and a large effect in the lower intestinal tract and heart disease (Iqbal *et al.*, 2011).

Previous studies on the same medicinal plants have also been documented from other regions. Methanolic extracts of *Dovyalis abyssinica* has been reported to be effective against *Colletotrichum musae* fungi with a zone of inhibition of 11.7 mm but methanolic extract of *solanum incanum* did not produce any zone of inhibition against the same fungi (Bazie *et al.*, 2014). The crude ethanol extract of *solanum incanum* showed high antibacterial activity (26 mm) against *Staphylococcus aureus* (Indhumathi and Mohandass, 2014). This was attributed to the various phytochemical constituents present in the crude ethanolic extracts. They were identified to have alkaloids, flavonoids, phenols, carbohydrates, tannins, triterpenoids, glycosides, steroids, resins and saponins. The study was in agreement with the present study that has proved to have the same phytochemicals.

4.0 CONCLUSION

The study has revealed that the two medicinal plants have various phytochemicals that contribute to the observed antifungal and antibacterial activities. This justifies their ethnobotanical applications in management of various ailments.

5.0 RECOMMENDATION

Further work should be done to isolate, characterize and elucidate the chemical structures of the bioactive compounds from the two medicinal plants. Considering the importance of these plants, they may be cultivated for more extensive scientific investigations that could reveal new potent antimicrobial agents.

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