RESEARCH ARTICLE OPEN ACCESS

Phytochemical evaluation, Antibacterial and Antifungal activity of Scadoxusmultiflorus

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Abstract:

Scadoxusmultiflorus popularly known as blood lily. Medicinal plants present a wide range of potentially phytochemical compounds that contain many useful properties, including anticancer, enzyme inhibition, anti-inflammatory, antifungal, and antibacterial. The present study aimed to focus on the *in vitro* antibacterial, antifungal and phytochemical screening of chloroform extract of *Scadoxusmultiflorus*. *Scadoxusmultiflorus of c*hloroform extract conducted antibacterial and antifungal activity against clinical s*taphylococcus aureus*, *klebsiella pneumonia* and *aspergillus niger* by using disc diffusion method. The phytochemical screening showed positive results for triterpenes/steroids, alkaloids, anthraquinones, coumarins, flavonoids, saponins, tannins, and phenolic acids. Chloroform extract showed significant antibacterial and antifungal activity. From this study concluded that *Scadoxusmultiflorus* having antibacterial and antifungal activity. Antibacterial and antifungal activity of *Scadoxusmultiflorus* may be due to the secondary metabolites such as alkaloid, tannins, terpenoids, flavonoids, and phenol compounds.

Keywords **—*Scadoxusmultiflorus*, Extraction, Phytochemical screening, Antibacterial, Antifungal**

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I. INTRODUCTION

Plants are naturally to god gifts for the production of medicines. Its isolation and classification from medicinal plants provide a great aid in the preparation of new medicines for the treatment of many diseases and are of great therapeutic benefit [1, 2]. The plant extract, also known as a natural product. It was of great help in discovery in the field of chemical diversity due to the uncertain availability as either standardized extract or as a pure compound. According to pharmaceutical research, about 10 to 20% of plants used to treat infectious diseases such as cancer positively in health care. The classic example is the yew tree bark, which contains taxol primarily and used in ovarian cancer and breast cancer. Isolation or extraction of medicinal plants mainly produced one or several responsible substances [3, 4]. According to the World Health Organization study, about 80 per cent of people used conventional medicine to treat primary health care. In Asia, plants show a long history as medicine with human involvement in the environment.

Herbal medicines contain various novel and particular substances for the treatment of infectious and chronic diseases [5]. Antibiotics are one of our most effective tools in fighting bacterial infections and since their introduction have significantly improved the health-related quality of human life. These health benefits are, however, under threat in recent decades, as many widely used antibiotics have become less and less effective against certain diseases, not only because many of them develop

toxic reactions [6, 7]. For centuries man knew herbal medicines. Practitioners of traditional medicine have identified the therapeutic efficacy of many indigenous plants for several disorders. Antimicrobial properties of medicinal plants are increasingly recorded from various parts of the world [8]. For conventional treatments of 80% of the world's population, the World Health Organization reports that plant extracts of the active constituents used as conventional medicine. Knowledge of the importance of medicinal plants has increased in recent years. These plants drugs are readily available, inexpensive, effective, powerful, and rarely accompanied by side effects. Plants that have used for medical use for thousands of years are the most obvious starting point for new therapeutically effective drugs, recently, the used of medicinal plants has increased given the progress made in the chemotherapy sector. The suggested reasons for this are the use of medicinal plants as resources to extract active pharmacological agents [9].

Scadoxus multiflorus grows from a "rhizomatous bu lb," that is to say, a bulb that also produces rhizome s (modified, underground stems). It is also known as the 'Fireball' and 'Pin Cushion Lilly' in India and 'scarlet lily', 'football lily' 'red cape lily', 'hood lily', 'blood flower' and 'powder puff lily' elsewhere. Scadoxus was named by Rafinesque, to mean glorious umbel. However the synonym genera' Haemanthus' is derived from Greek and refers to red coloured flowers refers to an innumerable number of flowers.

The leaves and flowers that appear together. The leaves ' bases, stalks or petioles, are tightly woven together to form a 5–60 cm (2–24 in) long pseudostem or false stem. The flowers are produced in a 12 - cm long umbel on a leafless stem. Both the scape and the pseudostem frequently painted with stripes of reddish to dark purple [10, 11]. The genus *Scadoxus* contains alkaloid-rich, actively toxic plants. All parts of this plant are poisonous due to a toxic substance called lycosine and some other alkaloids present in the plant. Two species *Scadoxusmultiflorus* and *Scadoxuscinnabarinus* are known to be used in Cameroon, Gabon, Angola and the Central African Republic in conjunction with several other plants, as an arrow poison. In Guinea and northern Nigeria, the bulbs used to make a fishing poison. The bulb also used to treat dropsy, scabies and poorly healing wounds [12].The inhibitory of two structurally and functionally identical enzymes inhibited by the novel plant protein isolated from *Scadoxusmulttiflorus*, xylanase and α-amylase (XAIP) bulbs: xylanase and α-amylanase.

Scadoxusmultiflorus is widely distributed in tropical Africa from Senegal through to Ethiopia, Gambia and Somalia; to Southern Namibia and South Africa, as well as to tropical Arabia, Transvaal and Natal and Seychelles (Saudi Arabia, Yemen, and Oman). This is naturalized in the Mexican and Indian Oceans of the Chagos Archipelagos. Lowland, medium-level, and mountain pastureland, woodlands and open secondary woods, woods with savannas, forest galleries, and coastal water habitat and ecologies.*scadoxusmultiflorus* prefers shade and is often found under trees, and it is winter-dormant and flowers in early summer. Butterflies are among the few insects that can see red, a characteristic that they share with birds and mammals. Such beautiful insects can be well suited to the orange or red flowers of *scadoxusmultiflorus*. Leurs small bunny floral clusters secrete energy-rich nectar at the bottom of the floral tube, where it can be accessed only to butterfly's siphon-like mouth (Fig.1).

Figure1. Leaves of *Scadoxusmultiflorus*

Plant Description
Taste: Bitter
Colour: green
Odour: characteristic
Perennial herb, growing from a large bulb. The plants die back every year and usually flower before the leaves fully develop. A single spherical inflorescence, containing up to 150 individual flowers, is borne on a fleshy stem.

Scientific classification

Kingdom: Plantae
Clade: Tracheophytes
Clade: Angiosperms
Clade: Monocots
Order: Asparagales
Family: Amaryllidaceae
Subfamily: Amaryllidoideae
Genus: *Scadoxus*
Species: S. Multiflorus
Binomial name: *Scadoxusmultiflorus*
English: Common fireball, Blood lily
Finnish: Loistotupsu
French: Ail rouge

Synonyms:

- *Amaryllis multiflora (Martyn) Tratt.*
- *Haemanthusabyssinicus Herb.*
- *Haemanthusandrei De Wild.*
- *HaemanthusarabicusM.Roem.*
- *Haemanthusarnoldianus De Wild. &T.Durand*
- *Haemanthusbequaertii De Wild.*

Habitat
A "rhizomatous bulb," namely a bulb that produces rhizomes (modified, sub-ground stems) is a product of scadoxusmultiflorus. The leaves and flowers can be produced together. The leaf bases, the stems or petioles are tightly bound in the shape of a 5-60 cm (2-24 in) long pseudostem or fake stem. The flowers are produced in an umbel, 12 – 75 cm (5-30 in) wide, over a leafless stalk (scape). The pseudostem, as well as the scape, are often outlined in reddish-brown violet spots. With 10 to 200 individual flowers, the umbel of flowers is more or less globe-like. Usually, each flora is 15-45 mm (0.6-1.8 inches) long with a stem (pedicel). The tepals, the endurance and the style, are fading to rose, scarlet. Tepals have their bases fused to form a 4–26 mm long cylindrical tube; their free ends are 12–32 mm (0.5–1.3 in) long, healthy and stretching. The fruit consists of a berry, 5–10 mm in size.

Cultivation and propagation: *scadoxusmultiflorus*is grown in containers or in the soil in which the environment is sufficient as ornamental plants for its brilliants coloured flowers. It is a winter-dormant, not frost-hardy plant and needs to be maintained at the minimum of 10 ° C.

Plant medicinal uses
These plants used to treat dropsy, scabies and poorly healing wounds. Bulbs reported as being very poisonous. The juice of *scadoxusmultiflorus* is supposed to produce dangerous, swelling of the lips and tongue, salivation, nausea, vomiting and diarrhoea. The leaves appear to have the same toxic effects as the bulb. The bulb used for, cough and snakebite; pounded leaves for diarrhoea and dysentery; roots powder for wounds, ulcers; boiled root infusion against child cough. This plant extract used in conjunction with several other plants an arrow poison, the bulbs used to make, a fishing poison; also used to treat dropsy, scabies and poorly healing wounds.

II MATERIALS AND METHODS

A. Collection and identification of *scadoxusmultiflorus*
The plant specimen for the study *scadoxusmultiflorus* collected from Thrissur – Kerala from June to September. Healthy plants were selected carefully. The plant parts were separated

B. Processing of Plant material (*scadoxusmultiflorus*)
The collected plant material was washed with tap water for 3 times and sterilized by spraying with 70% alcohol. The sterilized plant material was shade dried at room temperature to avoid chemical changes and frequently observed for any fungal contamination as the plant material rich in water content. When the plant material was dried entirely,

it is subjected to prepare a fine powder. The dried plant leaves were powdered using a mechanical grinder and passed through 60mesh sieve to get the powder of desired coarseness. Powder material preserved in an airtight container. The fine powder is collected and used for extraction of the crude drug in different solvents from non-polar to polar by Soxhlet extraction method.

C. Extraction of phytoconstituents by soxhlet extraction

The extraction procedure for the isolation of crude drug from plants has been practised for a long time. The precise mode of extraction naturally depends on the texture and water content of the plant material extracted and on type of substance that is isolated. Usually, the crude extract is taken from the soxhlet apparatus with the help of non-polar to polar solvents (Fig.2).

Figure 2. Soxhlet apparatus

This apparatus mainly consists of three parts, round bottom flask in which the solvent is taken, main jar in which material from which the compounds to be extracted and kept loaded and condenser in which condensation of vapours of solvents takes place. 100 g of the powder of plant material from which the extract has to be taken and packed into soxhlet main jar. The solvent is transferred into the round bottom flask and extract condensation under reduced pressure and controlled temperature of 60-80°C set to boil through the regulated heating mantle. The vapour of the solvent pass-through drive tubes enters the condenser through the main jar and gets condensed where there is the continuous flow of water in the condenser. The condensed solvent falls back on the packed material in the main jar before collecting in the jar. The collection and extraction of material take place simultaneously in the main jar as seen by the colouring of the solvent as the compound of material get dissolved in the solvent. Thus, the crude extract of the plant material obtained for the isolation of all phytoconstituents from the plant requires a relatively large volume of organic solvents such as Chloroform with solvent plant ratio used is 10:1, it usually takes 7-8 hours for complete extraction. The solvent evaporates, and finally, it yields brown/green/ waxy extract and stored in the refrigerator for further usage.

D. The Quantitative evaluation of primary and secondary metabolites of *Scadoxus multiflorus*[12.]

The plant extracts obtained after each successive solvent extraction qualitatively tested for the presence of various phytochemicals. The preliminary phytochemical screening was carried out by the Method by described as Harborne (1991) and Kokate (1995)

Phytochemical Screening

Test for Alkaloids

- Dragendroff's test: The extract treated with Dragendroff'sreagent(potassium bismuth iodide solution).The orange-brown precipitate formed, which indicate the alkaloids occurrence.
- Mayer's reagent: The plant extract treated with Mayer's (potassium mercuric iodide solution) reagent. The precipitate formed, which shows the presence of alkaloids.
- Wagner's reagent: The extract was treated with Wagner's reagent (iodide and potassium triiodide solution).Reddish-brown Precipitate was formed, and alkaloids are present.

Test for Glycosides

- Bontrager's test: To the extract, add dilute Sulphuric acid and filtered. The filtrate was extracted with little chloroform layer was separated and add an equal volume of dilute ammonia. The red colour observed in ammonical layer confirms the presence of glycosides.

Test for saponin glycoside

- Foam test: Shake the extract with water. The foam was produced, which indicates the presence of saponins.

Test for Tannins and Phenolic compounds

- Ferric chloride test: To the aqueous extract few drops of ferric chloride solution were added. The dark black colour formed which shows the presence of tannins and phenolic compounds.
- Bromine water test: To the aqueous extract is treated with bromine water— discolouration of bromine Waterindicates the presence of tannins and phenolic compounds.
- Potassium permanganate test: To the aqueous extract is treated with dilute Potassium permanganate. Discolouration of the solution indicates the presence of tannins and phenolic compounds.

Test for reducing sugars

- Benedict's test: 0.5ml of extract solution 1ml of water 5 to 8 drops of Fehling's solution added. No red brick precipitate which shows an absence of reducing sugars.

Test for Amino acids

- Ninhydrin test: The aqueous extract heated with 5%ninhydrin solutions on boiling water bath for 10 min. No purple colour formed and shows the presence of amino acid.
- The aqueous extract is treated with solution sodium hydroxide and lead acetate solution and boiled. No black precipitate is formed and shows the presence of amino acid.

Test for flavonoids

- Shinoda test: To the methanol extract, add potassium hydroxide solution and then 10% ammonia. Yellow colour Precipitate formed and indicated the presence of flavonoids.
- To the ethanol extract, add a few drops of Lead acetate solution. Yellow colour Precipitate formed, which shows the presence of flavonoids.

Test for terpenoids.

- 1.4gm of the extract was treated with 0.5ml of acetic anhydride and 0.5ml of chloroform and added a concentrated solution of sulphuricacid.No Red violet colour was obtained, which indicates the absence of terpenoids.

Test for steroids

- Libermann- BuchardTest: To extract added chloroform solution a few drops of acetic anhydride and 1ml of concentrated sulphuric acid was added through the side of the test tube and set aside for a while. Brown ring formed at the junction who shows the presence of steroids.
- Salkowski Test: To the extract add chloroform solution a few drops of concentrated sulphuric acid added shaken and allowed to stand. Greenish fluorescence was formed and confirmed the presence of steroids.
- Libermann's Reaction: Mix 3ml of an extract with 3ml of acetic anhydride, heat and cooling. Add a few drops of Concentratedsulphuricacid. The blue colour was formed and indicated the presence of steroids.

E. Antibacterial activity of *Scadoxusmultiflorus* against pathogenic microorganisms

Strain, Culture media and Sterile discs

Extract and standard drug were conducted for bacterial activity against clinical Staphylococcus aureus, Klebsiella pneumonia and Aspergillus spp. Microbial cultures from Tiruchirappalli, Tamil Nadu Government Medical College. Muller-Hinton agar media of Himedia Pvt were media used for the microbial research. Mumbai, India. Microbial cultures procured from government medical college from Tiruchirappalli, Tamil Nadu. Media used for

the microbial test was Muller-Hinton agar media of HimediaPvt. Bombay, India.

Antibacterial activity

Antibacterial activities of chloroform extract were studies by using disc diffusion method. Escherichia coli, Staphylococcus aureus, inoculums prepared by using nutrient broth media. Double strength sterile Mueller-Hinton agar media were prepared by autoclaving 7.6 gm in 100ml. Inoculate the test microorganisms on the Mueller-Hinton agar plates by using sterile cotton swabs. The chloroform extract of *Scadoxusmultiflorus* placed on sterile discs. Discs were dried aseptically under laminar airflow to remove solvents. Dried discs placed on the surface of culture inoculated Mueller-Hinton agar plates and plates incubated at 37˚C for 24hr. Antibacterial activity evaluated by using media zone reader [13].

Antifungal activity

Antifungal activity of *Scadoxusmultiflorus*chloroform extracts studied by using disc diffusion method. Aspergillusspp, the inoculum was prepared by using potato dextrose broth. Potato dextrose agar media prepared by autoclaving 3.9 gm. in 100ml. Inoculate the test microorganisms on the potato dextrose agar plates by using sterile cotton swabs. The chloroform extract of *Scadoxusmultiflorus* placed on sterile discs. Discs were dried aseptically under laminar airflow to remove the solvent. Dried discs placed on the surface of culture inoculated potato dextrose agar plates and the plates incubated at room temperature for 48hr. Antifungal activity evaluated by using media zone reader.

III RESULT AND DISCUSSION

A. Phytochemical screening

Phytochemical results are showed in the table 1. *Scadoxusmultiflorus* leaves have present the secondary metabolites of steroids, alkaloides, glycoside, flavonoids, tannins and phenolic compounds.

Table 1. Quantitative estimations of primary and secondary metabolites of *Scadoxusmultiflorus*

Chemical test	Chloroform extract
Test for Alkaloids	
Dragendroff's test	+
Mayers' reagent's	+
Wagner's reagent	+
Test for Glycosides	
Bontrager's test	+
Test for saponin glycoside	
Foam test	+
Test for Tannins and phenolic compounds	
Ferric chloride test	+
Bromine water test	+
KMnO4test	+
Test for reducing sugars	
Benedict's test	-
Test for amino acids	
Ninhydrin test	+
NaOH& lead acetate test	+
Test for flavonoids	
Shinoda test	+
Lead acetate test	+
Test for Terpenoid	-
Test for steroids	
Libermann-Buchard test	+

Salkowski Test	+
Libermann's Reaction	+

B. Antibacterial activity of *Scadoxusmultiflorus*chloroform extract.

Antibacterial activity of *Scadoxusmultiflorus*chloroform extract and standard drug against pathogenic microorganisms *Staphylococcus aureus* and *Klebsiella pneumonia* (Table 2 and fig.3).

Table 2. Antibacterial activity of *scadoxusmultiflorus*extract and standard drug against clinical *Staphylococcus aureus* and *Klebsiella pneumonia*

Formulations/Standard drug	Zone of Inhibition (mm)	
	Staphylococcus aureus (n=2)	*Klebsiella pneumoniae* (n=2)
Amikacin	28±5mm	26±5mm
Chloroform extract	21±5mm	18±5mm

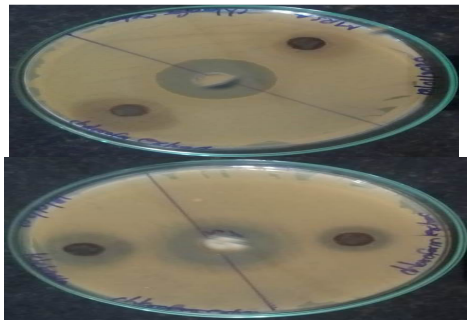


Figure 3. Antibacterial activities of *Scadoxusmultiflorus* extract and standard drug against clinical *Staphylococcus aureus* and *Klebsiella pneumoniae*

Scadoxusmultiflorus extract and standard drug showing antibacterial activity against clinical pathogenic *Staphylococcus aureus*and *Klebsiella pneumoniae*

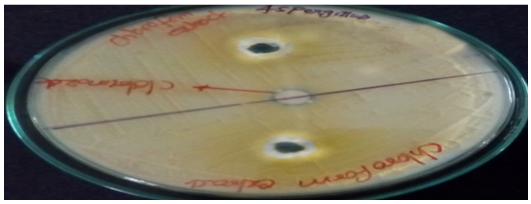
Antifungal activity of *Scadoxusmultiflorus*chloroform extract

Antifungal activity of *Scadoxusmultiflorus*chloroform extract and standard drug against pathogenic microorganisms *Aspergillus spp* (Table 3 and figure 4)

Table 3. Antifungal activity of *scadoxusmultiflorus* extract

Extract/Standard drug	Zone of Inhibition (mm)
	Aspergillus spp (n=4)
Chloroform extract	6±5
Clotrimazole	2±5

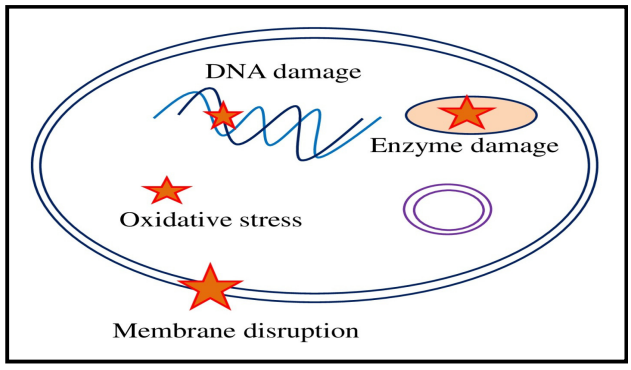
Figure 4. Antifungal activity of *Scadoxusmultiflorus* extract chloroform extract showing activity against human pathogenic *Aspergillus spp*



Scadoxusmultiflorus extract and standard drug showing antifungal activity against clinical pathogenic *Aspergillus spp*

The antibacterial activity of *Scadoxusmultiflorus*chloroform extracts due to may be phytoconstituents compounds such as alkaloids, saponins, tannins, flavonoids and steroids have been known to be biologically active. The *Scadoxusmultiflorus* metabolites generate the reactive pieces; these reactive species are responsible for the bacterial DNA alteration, membrane disruption, oxidative stress which cause the cell death (Fig.5)

Figure 5. Possible mechanism for antimicrobial activity of *Scadoxusmultiflorus* metabolites



IV CONCLUSION

Scadoxusmultiflorus is an herbaceous plant that is fleshy and has a wide bulb with a length of 25 cm and 8cm which occur during the rainy season. Trepen and/or steroids, alkaloids, anthraquinones, flavonoids, saponins, tannins, anthraquinones and phenolic acids were confirmed to be secondary metabolites in Scadoxusmultiflorus leaves. Results obtained from this study suggest that *Scadoxusmultiflorus*may be attractive for the 'drug hunters' as a potential agent for the management of infectious diseases against the human pathogens *Klebsiella pneumonia, Staphylococcus aureus* and *aspergillus spp.*

Acknowledge

The authors wish to acknowledge and thank the support of the Sanjo College of Pharmaceutical Studies, Centre for Biotechnology and Phyto Pharmacognosy Research, Coimbatore and Anna University, BIT Campus for providing all facilities necessary to carry out the work.

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