

# Phytochemical Screening and In vitro Evaluation of Antimicrobial Activity of *Kedrostis foetidissima* extract impregnated Face Mask

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## ABSTRACT

Leaf and stem ethanol extracts of *Kedrostis foetidissima* (Jacq.) Cogn. were screened for various phytochemicals present in it by adopting standard procedures. Phytochemical screening indicates the presence of alkaloids, flavonoids, phenols, triterpenoids, steroids, tannins, cardiac glycosides and saponins. The aforesaid extracts were coated over face mask and screened for its antimicrobial activity towards common sweat bacterium *Coryne bacterium* and *Candida albicans* (fungi) using AATCC147 method. The fabricated face mask showed significant and remarkable antimicrobial activity against microbes when compared with standard. The present study reveals *Kedrostis foetidissima* extract to be a promising source for the fabrication of antimicrobial face masks.

**Keywords:** *Kedrostis foetidissima*, *Coryne bacterium*, *Candida albicans*.

## 1. INTRODUCTION

Plant-derived substances are of substantial interest owing to their multifaceted usage. Herbs are the copious biological source of medicine, conventional and modern, nutraceuticals, dietary supplements, folk medicines, mixture of active substances and excipients and chemical entities for synthetic drugs<sup>1</sup>. Ethno medicinal study deals with traditional medicines. From time immemorial mankind has been using herbal plants, organic materials as well as materials from the sea, rivers etc. for treating various ailments. Easily available herbs have been explored to a great extent for their medicinal properties. Various parts of plants like roots, leaves, bark, exudates etc. were also used as per its medicinal properties<sup>2</sup>. Recent reports says that of 119 compounds extracted from 91 plant species are considered to be vital and practicable drugs. Of these 77% of the drugs are traditional<sup>3</sup>. Densely populated nations experienced more bacterial infections which have more resistance to antibiotics. This has

necessitated the search for new antibacterial agents<sup>4</sup>. Weakened immunity has complicated due to bacterial infections in the cases of patients with chronic conditions, cancer, AIDS and who underwent transplantations<sup>5,6</sup>.

*Kedrostis foetidissima* (Jacq.) Cogn. traditionally called "Appakovai" in Tamil. This herb which grow around the fence and cattle feeds them ferociously. *Kedrostis* was traditionally used in the treatment of ailments like chest pain, asthma and infections in urinary tract, small pox, diarrhoea, skin allergies<sup>8</sup> and snake-bite<sup>9</sup>. Juice extracted from its leaf is effective in treating cattle's bloat<sup>10</sup> and cold in children<sup>11</sup>. All parts of *K.foetidissima* show inhibitory activity against the growth of gram positive and gram negative bacterial strains<sup>12</sup>. Our present study is intended to screen the phytochemicals present in various stem and leaf extracts of *Kedrostis* and the antimicrobial activity of extract impregnated face masks.

## 2. MATERIALS AND METHODS

### 2.1. Collection of Plant Materials

*Kedrostis foetidissima* is collected from Aliyar hills, near Pollachi. The voucher specimen has been submitted to Botanical Survey of India, Tamilnadu Agri. University, Coimbatore, Tamilnadu. It was identified as *Kedrostis foetidissima* (jacq.) cogn (= *Trichosanthes foetidissima* Jacq.) Cucurbitaceae family by Dr. P. Satyanarayanan, Scientist-D (BSI/SRC/5/23/2010-11/Tech.-1309). The collected plant materials were washed thoroughly to remove mud particles, separated and then shade dried. The stem and leaves were crushed and stored in hermetically sealed containers for further use.

### 2.2. Extraction of plant Materials.

The leaves and stem (100 g) of *Kedrostis foetidissima* (jacq.) cogn., were first defatted with petroleum ether. Ethanol (ETOH), Chloroform (CF), Ethyl Acetate (EA), Petroleum Ether (PE) extract of this plant is prepared by refluxing (6h) 100g of pulverized leaves and crushed stem with 1 liter of each solvent mentioned above. All extracts were concentrated to get dry residues. The dry extracts were refrigerated for future use.

### 2.3. Phytochemical Screening

The phytochemical tests were carried out for the leaf and stem extracts using standard procedures to identify the primary and secondary metabolites<sup>13-15</sup>

#### Test for Alkaloids

##### Hager's test

A fraction of the extract was treated with Hager's reagent [Saturated aqueous solution of Picric Acid] and observed for the formation of reddish colour precipitate.

##### Wagner's test

A fraction of extract was treated with 3-5 drops of Wagner's reagent [1.27g of iodine and 2g of potassium iodide in 100ml of water] and observed for the formation of reddish brown precipitate (or colouration).

##### Mayer's test

A fraction of the extract was treated with Mayer's Reagent [1.36 g of Mercuric Chloride and 5g of Potassium Iodide in 100ml of water] and observed for the formation of cream colour precipitate (or colouration).

#### Dragendroff's Test

A fraction of the extract was treated with Dragendroff's reagent [solution A: Bismuth nitrate (0.17g) in acetic acid (2ml) and water (10ml); Solution B: Potassium Iodide (4g) in acetic acid (10ml) and water (20ml); Mixture of A and B and diluted to 100ml with water]. Formation of red precipitate indicates the presence of alkaloids.

#### Test for Phenols

##### Ferric chloride test

A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for the formation of deep blue or black colour.

##### Lead acetate test

A fraction of the extracts was treated with 10% lead acetate solution and observed for formation of white colour precipitate indicates the presence of phenols.

#### Test for Flavonoids

##### Ferric chloride test

A fraction of the extracts was treated with few drops of ferric chloride solution. Formation of intense green colour indicates the presence of flavonoids.

##### Lead acetate test

A fraction of the extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

##### Alkaline reagent test

A fraction of the extract was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow colour, which becomes colorless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

##### Shinoda

A fraction of the extract was treated with Magnesium turnings and Conc. HCl was added drop wise, pink, scarlet, crimson red or occasionally green to blue colour appears after few minutes indicates the presence of Flavonoids.

#### Test for Terpenoids and Sterols

##### Liebermann – Burchard test

A fraction of the extract was treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added along

the sides of the test tube. A brown ring at the junction of two layers with green upper layer shows the presence of steroids and formation of red colour indicates the presence of triterpenoids.

#### **Salkowki's test**

A fraction of the extracts was treated with 1ml chloroform followed by few drops of concentrated sulphuric acid. Shaken well and allow to stand for 5 min. Red colour in the lower layer indicates the presence of sterols and the formation of yellow coloured lower layer indicates the presence of triterpenoids.

#### **Test for Cardiac Glycosides**

##### **Keller Kelliani's test**

A fraction of the extracts was treated with 2ml of glacial acetic acid and a drop of ferric chloride solution in a test tube. This was carefully underlayered with 1ml concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form.

#### **Test for Tannins**

##### **Braymer's test**

Two ml extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution.

#### **Test for Saponins**

##### **Foam test**

Two ml extract was diluted with 6ml water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

## **2.4. Antimicrobial Activity**

### **Fabrication of medical face masks-pretreatment procedure**

Commercially available face mask was purchased from a pharmacy in Coimbatore. The piping materials, ties and ear loops were removed and washed several times with distilled water. The washed mask was then dried in hot air oven maintained at a temperature of 60° C for 5 min, after which it was cut into small circles of 1cm diameter. The samples were stitched around to avoid separation of layers. Four such samples were then impregnated with the leaf ethanol extract (KFLEOH-A), stem ethanol extract (KFSEOH-B) and standard CIFRAN-C. A blank mask was also studied and

the specimen was assigned the sample code BLANK-D.

### **Impregnation of extract onto the face masks**

Exactly 100µg leaf ethanol extract (KFLEOH –A) was dissolved in 1ml distilled water and sonicated for 30min to get a uniform mixture. The pretreated mask samples were loaded with the leaf ethanol extract by spraying the solution uniformly over it. Then the samples were allowed to dry in air. Same procedure was adopted for stem ethanol extract (KFSEOH -B), standard – (CIFRAN )–C and BLANK- D. The fabricated samples were used for the assessment of antimicrobial activity against *Coryne bacterium* and *Candida albicans* (fungi). The fabricated face masks are assigned the sample codes KFLEOH(A), KFSEOH(B), CIFRAN-STANDARD(C) and BLANK MASK (D).

### **Determination of Antimicrobial Activity of Facemask by Disc Diffusion method**

#### **Microbial stains tested**

Antimicrobial activity of mask coated with ethanol extracts and Cifran were tested against *Coryne bacterium* and *Candida albicans* (fungi) according to AATCC147 standard by disc diffusion method.

#### **Preparation of Inoculums**

Fresh nutrient broth from sealed slant culture is used to prepare the inoculums for this experiment. The turbidity was adjusted to standardize the culture to that of McFarland standards. This was achieved by adding sterile saline or broth<sup>16</sup>

#### **Preparation of Sterile Swabs**

A cotton wool swab on an applicator made of wood was equipped and packed in culture tubes. The tubes were autoclaved for sterilization.

#### **Sterilization of forceps**

The forceps were sterilized by dipped in alcohol and introducing into the flame.

## **EXPERIMENT**

### **Disc Diffusion Method**

Dipping of sterile swab into the standardized inoculums and inoculating onto the aseptically prepared plates, excess of inoculums was removed by pressing and revolving the swab decisively alongside of the culture tube over the liquid level. Then streaking the swab throughout the surface thrice revolving the plate by an angle of 60°C was done after each application. At last

the swab was passed around the edge of the agar surface. At room temperature, the inoculums were dried with the lid closed.

All Petri dishes were split into four parts. Samples, KFLEOH(A), KFSEOH(B), CIFRAN-STANDARD(C) and BLANK MASK (D) were placed in each part of the Petri dish using sterile forceps. The Petri dishes were refrigerated at 4° C for an hour for diffusion. After that they were incubated for 24 hours at 37 ° C. The zone of inhibition produced by different samples was then measured. The mean value of two diameters measured in opposite directions of each zone of inhibition was then recorded.

### 3. RESULTS AND DISCUSSION

#### Phytochemical screening

The eight solvent extracts of *Kedrostis foetidissima (jacq.)cogn* were screened for the phytochemical constituents present in them by standard procedure. The ethanol extracts of stem and leaves shows high precipitation of alkaloids, phenols, flavonoids, triterpenoids and steroids, moderate precipitation of saponins and low precipitation of tannins and cardiac glycosides (Table-1).

Chloroform stem extract shows high precipitation of alkaloids, phenols, triterpenoids and steroids. It also shows moderate precipitation of flavonoids, low precipitation of tannins and absence of saponins and cardiac glycosides. Chloroform leaf extract shows high precipitation of phenols, triterpenoids and steroids, moderate precipitation of alkaloids, flavonoids low precipitation of tannins and saponins and cardiac glycosides.

Petroleum ether stem extracts shows high precipitation of triterpenoids and steroids, low precipitation of alkaloids and absence of phenols, flavonoids, tannins, saponins and cardiac glycosides whereas leaf extract shows moderate precipitation of triterpenoids and steroids, low precipitation of alkaloids, and absence of flavonoids and tannins.

Ethyl acetate stem extract shows moderate precipitation of flavonoids, triterpenoids and steroids, low precipitation of phenols and absence of tannins saponins and cardiac glycosides. Ethyl acetate leaf extract shows moderate precipitation of flavonoids, triterpenoids and steroids, low precipitation of and absence of phenols, tannins saponins and cardiac glycosides.

**Table 1: Phytochemical Analysis of *Kedrostis foetidissima (jacq.)cogn***

PHYTOCHEMICALS/ TESTS	LEAF				STEM			
	KFPE	KFEA	KFCHLF	KFEOH	KFPE	KFEA	KFCHLF	KFEOH
<b>ALKALOIDS</b>								
i) Hager's	+	-	++	+++	+	-	+++	+++
ii) Wagner's	+	-	++	+++	+	-	+++	+++
iii)Mayer's	+	-	++	+++	+	-	+++	+++
iv)Dragendroff's	+	-	++	+++	+	-	+++	+++
<b>PHENOLS</b>								
i) FeCl <sub>3</sub>	-	-	+++	+++	-	+	+++	+++
ii) Lead Acetate	-	-	+++	+++	-	+	+++	+++
<b>FLAVONOIDS</b>								
i) FeCl <sub>3</sub>	-	++	++	+++	-	++	++	+++
ii) Lead Acetate	-	++	++	+++	-	++	++	+++
iii)Alkaline reagent	-	++	++	+++	-	++	++	+++
iv)Shinoda	-	++	++	+++	-	++	++	+++
<b>TRITERPENOIDS and STEROIDS</b>								
i)Liebermann	++	++	+++	+++	+++	++	+++	+++
ii)Salkowski	++	++	+++	+++	+++	++	+++	+++
<b>CARDIAC GLYCOSIDES</b> Keller-Killani	-	-	-	+	-	-	-	+
<b>TANNINS</b> Braymer's	-	-	+	+	-	-	+	+
<b>SAPONINS</b> Foam test	-	-	-	++	-	-	-	++

KFPE- Petroleum Ether, KFACET- Acetone, KFCHLF- Chloroform, KFEOH- Ethanol

+++ High Precipitation, ++ Moderate Precipitation, + Low Precipitation and – Negative Result.

Phytochemicals in plants are responsible for its mode of metabolic activity. Review of literature on the specific activity and mechanism of various phytochemicals reveal most of the secondary plant metabolites to possess significant activity (Table-2). Almost all phytochemicals present in *Kedrostis foetidissima* show antimicrobial activity. So it is worthy to investigate the antimicrobial activity of this species.

### 3.2. Antimicrobial activity

Fabricated mask samples were tested for their antimicrobial activity against *Coryne bacterium* and *Candida albicans* according to AATCC147 standard using disc diffusion method. According to AATCC147 interpretation, for a successful result, there should be no bacterial growth observed under and on the fabricated samples and absence of leaching of extract. In the present study after the incubation time, a clear zone of inhibition was noted against the organism by the test samples (Table 3 and Figure -1).

The results of the present study shows a significant anti microbial activity since there was no bacterial growth under and above the test samples as mentioned by AATCC147 standard. The inhibitory activity of KFLEOH (A- 20 mm) and KFSEOH (B-21 mm) fabricated mask towards *Coryne bacterium* were close to that of the standard CIFRAN(C-22 mm). The zone of inhibition was comparatively more for mask fabricated with stem ethanol extract. In case of *Candida albicans*, inhibitory activity of mask fabricated with KFLEOH (A-16 mm) is comparatively more than that of the mask coated with KFSEOH (B-15 mm). The samples were certified for its antimicrobial activity by KMCH college of Pharmacy, a college approved by the Pharmacy Council of India.

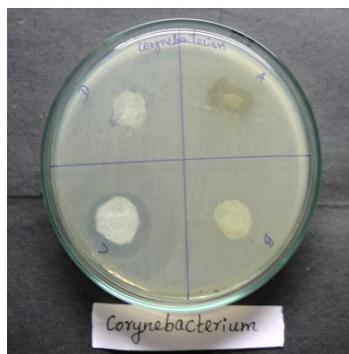
The significant antimicrobial activity of fabricated mask is attributed to the existence of phytochemicals like alkaloids, flavonoids, phenols, triterpenoids, steroids, tannins, cardiac glycosides and saponins in the impregnated ethanol extracts as exemplified from the phytochemical screening results.

**Table 2: Mechanism of action of some phytochemicals**<sup>17-30</sup>

Phytochemicals	Activity	Mechanism of action
Alkaloids	Antimicrobial	Intrudes inside cell wall and parasite's DNA Hinders discharge of prostaglandins and autocoids.
	Antidiarrhoeal	Have anti-oxidating property, diminishes nitrate production, Inhibits transmission of sucrose from stomach toward small intestine, reduces the glucose support to the helminthes, act upon central nervous system leading to paralysis
	Anthelmintic	
Polyphenols and Tannin	Antimicrobial	Binds to adhesions, enzyme hindrance, substrate destitution, intricate through cell wall, membrane separation, complexation of metal ion.
	Antidiarrhoeal	Improves the resistant power of s intestinal and diminishes secretion, normalizes distracted water transport through the mucosal cells and decrease intestinal movement, diarrhea induced by heat-labile enterotoxin is controlled, possesses astringency.
	Anthelmintic	Due to the formation of protein complexes in cud, supply of digestible proteins by animals is increased, impedes energy formation, decreases G.I. metabolism
Flavonoids	Antimicrobial	Intricate through cell wall, Hinders discharge of prostaglandins and autocoids,
	Antidiarrhoeal	Contractions due to spasmogens is diminished, Normalizes distracted water transport through the mucosal cells, GI discharge of acetylcholine is hindered.
Terpenoids and essential oils	Antimicrobial	Membrane separation
	Antidiarrhoeal	Hinders discharge of prostaglandins and autocoids.
Glycosides	Antidiarrhoeal	Hinders discharge of prostaglandins and autocoids.
Steroids	Antidiarrhoeal	Improves intestinal absorption of Sodium ion and water.
Saponins	Antidiarrhoeal	Hinders histamine discharge in vitro
	Anticancer	Has membrane permeability.
	Anthelmintic	Generation of vacuoles and fragmentation of teguments.

**Table 3: Zone of inhibition exhibited by facemasks impregnated with ethanol extracts of *Kedrostis foetidissima (jacq.)cogn***

ORGANISM	Zone of inhibition (in mm)			
	A (KFLEOH) 100µg/ml	B (KFSEOH) 100µg/ml	C STANDARD (CIFRAN) 100µg/ml	D (BLANK)
<i>Coryne bacterium</i>	20	21	22	13
<i>Candida albicans</i> (fungi)	16	15	18	12



***Coryne Bacterium***



***Candida albicans***

**Fig. 1: Zone of inhibition of *Coryne bacterium* and *Candida albicans***

The present study is a preliminary study to assess the application of antimicrobial plant extracts in the fabrication of medical textiles. As a sample study, face masks were tested for its antimicrobial potential. Further study on isolated compounds is in progress in our laboratory. The results of the study are promising and demonstrate the successful use of herbal extracts in fabrication of antimicrobial textile materials.

#### 4. CONCLUSION

The results of present evaluation indicates that *Kedrostis foetidissima (Jacq.) Cogn* is a potent source of phytochemicals with significant antimicrobial activity towards *Coryne Bacterium* and *Candida albicans*. *Coryne bacterium* is the etiological agent of diphtheria, causative of upper respiratory tract illness and common in human sweat. *Candida albicans* can cause a fungal infection known as candidiasis or moniliasis, It can cause an infection in mouth known as oral thrush which may cause a painful condition called burning mouth syndrome. Face masks coated with *Kedrostis* extract can probably act as a significant antimicrobial agent in the inhibition of upper respiratory tract illness caused by *Coryne bacterium*, skin infection and oral thrush caused by *Candida albicans*.

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