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Research Article

Antimicrobial Activity and Phytochemical Screening of Methanol Extract of *Chlorophytum kolhapurens* and *Chlorophytum baruchii*

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ABSTRACT

Chlorophytum borivilianum (Asparagaceae) is an important medicinal plant known as 'Safed musli' and is used in herbal drug industries. In present study we used two species of *Chlorophytum* viz., *Chlorophytum kolhapurens* and *Chlorophytum baruchii* for its potential antibacterial activity. Crude methanol extracts as well as active compound methanol extract of *Chlorophytum* species were screened for *In vitro* antimicrobial activity and also to evaluate the phytochemicals present in the species. Disc diffusion method was used to study the antimicrobial activity against bacteria *Staphylococcus aureus, Escherichia coli, Enterococcus fecalis, Pseudomonas aeruginosa and Proteus vulgaris*. Qualitative phytochemical analysis of *Chlorophytum* species confirmed the presence of alkaloids, carbohydrates, tannins, proteins, amino acids, saponins and steroids. The phytochemical screening was also confirmed by HPTLC analysis for saponins. *Chlorophytum kolhapurens* showed highest activity against *Proteus vulgaris*, whereas *Chlorophytum baruchii* showed highest activity against *Escherichia coli*. In the present study, we concluded that the crude methanol extract of *Chlorophytum* species showed excellent antimicrobial activity as compared to active compound extract, and it is attributed due to the presence of phytochemicals.

Keywords: *Chlorophytum kolhapurens*, *Chlorophytum baruchii*, Antibacterial activity, Pharmacognosy

INTRODUCTION

Plants have been used as medicine as long as history is concerned. Medicinal plants contain substances for therapeutic purposes as well as a synthetic precursor for useful drugs. Out of 20,000 medicinal plants of the world, India accounts about 15 percent (3000 – 3500) medicinal plants, growing in different climatic regions of the country[1]. It is estimated that 80% of the people worldwide rely on herbal medicines and on traditional health care system[2]. Plants have been the richest source of drug of traditional medicine, folk medicine, modern medicine, food supplements, pharmaceutical intermediates, neutraceuticals and chemical entities for the use in synthetic drugs[3]. In Hindu culture, in 'Rigveda', the earliest use of plants as medicine has been found which is supposed to be written in 4500-1600 B.C. It is considered to be the oldest repository of human knowledge. Ayurveda is considered as foundation of medicinal science of Hindu culture[4].

The numbers of drug resistant microorganisms with reduced susceptibility to various antibiotics are increasing. This has lead to increased use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection[5-8]. In developing countries, synthetic drugs are not only expensive and inadequate in treatment of diseases but also often with adulterations and side effects. Therefore, new infection-fighting strategies are required to control microbial infections[9]. One of the methods to reduce the resistance to antibiotics is by using antibiotic resistance inhibitors from plants[10,11]. Plants produce chemical compounds known as 'Phytochemicals' during secondary metabolism which have the ability to inhibit the growth of microorganisms. These phytochemicals in pharmaceutical industries are now used to make drugs against microorganisms either directly, as precursor or as a lead compounds. *Chlorophytum* Ker Gawl. (Asparagaceae) contain 217 species, six subspecies and eight varieties which are distributed throughout the tropical and subtropical parts

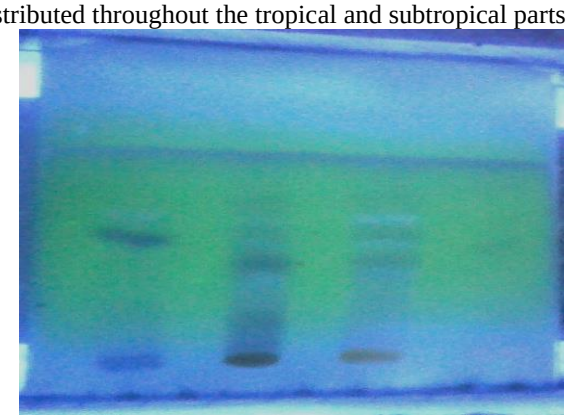

Figure 1: TLC of *Chlorophytum*

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Table 1: Physical characteristic of *Chlorophytum*

Physical characteristic	*Chlorophytum kolhapurens*	*Chlorophytum baruchii*
Color	Red-brown	Creamy-brown
Odour	No odour	No odour
Taste	Characteristic	Characteristic
Consistency	Porous	Porous
Percentage yield	10.2%	11.5%

of the world. *Chlorophytum* is a small perennial herbs mostly found in rain fed areas of India and Nepal. In India 17 species of *Chlorophytum* are found wild in natural forest and hilly areas of Southern Rajasthan, North Gujarat, Western Madhya Pradesh, Maharashtra and Karnataka[12]. Species of *Chlorophytum* are popularly known as 'Safed musli' in Indian drug market. The roots of the plant contain 42% carbohydrates; 8-9% proteins; 3-4% fibers, 2-17 % saponins and 15-20% alkaloids. Its roots (tubers) are widely used for various therapeutic applications. It is used to cure physical illness and weakness, as an aphrodisiac, antidiabetic, antistress, immunomodulatory, anti-inflammatory, antioxidant, arthritis, anti-ageing, and increases immunity[13-16]. In present study, we analyzed the phytochemicals and evaluated the antimicrobial activities of *Chlorophytum kolhapurens* and *Chlorophytum baruchii*. The antimicrobial activities of the extracts were determined by disc diffusion method.

MATERIALS AND METHODS

Plant material

The whole plant of *Chlorophytum kolhapurens* and *Chlorophytum baruchii* were collected from natural population from Western Ghats of Maharashtra during rainy season. Collected species were identified and confirmed with the herbarium species at Botanical Survey of India (BSI), Pune, Maharashtra. The specimens were deposited as herbarium in BSI, Pune, Maharashtra.

Preparation of extracts

The tubers (roots) of the plants were washed thoroughly with running water to remove dirt. The tubers were than shade dried, powdered using blender and stored in air tight containers. Dried powder (5g) was extracted in Soxhlet reflux extractor with petroleum ether to remove lipids and fatty acids, ethyl acetate and chloroform to remove proteins and hexane to remove fats. The defatted powder was then extracted with methanol for 48 hours

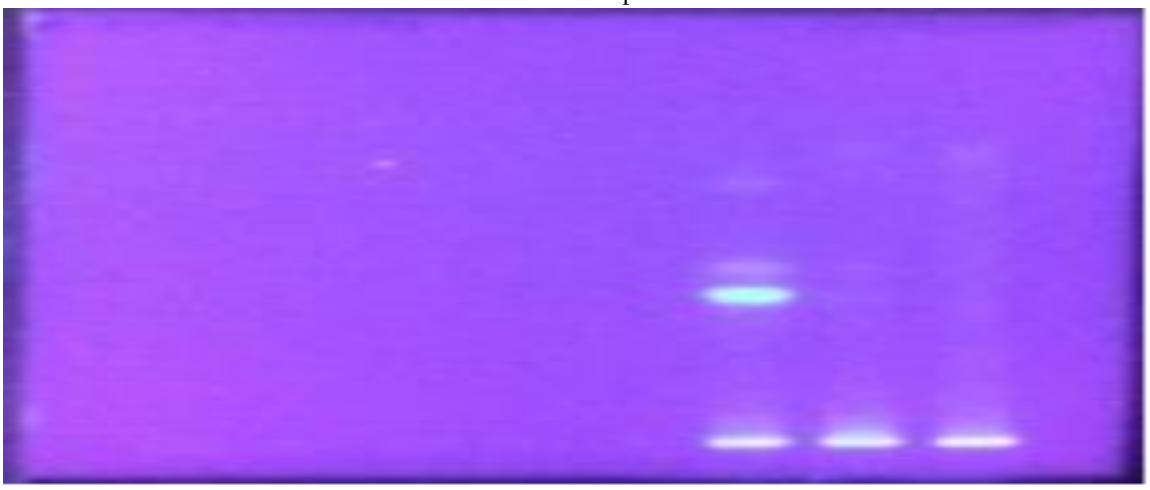

Figure 2a: Detection of saponins by HPTL

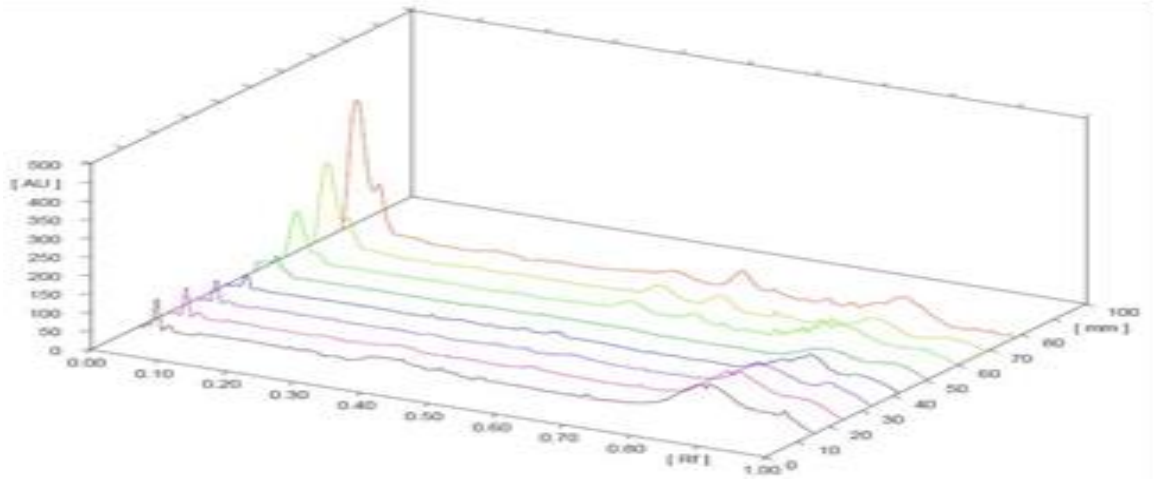

Figure 2b: HPTLC analysis: Wavelength 10-50: Standard Saponin, 60: *Chlorophytum borivilianum* (used for reference), 70: *Chlorophytum kolhapurens*, 80: *Chlorophytum baruchii*.

until the solvent turned colorless. The methanol extract was filtered through Whatmann no.1 filter paper in a Buchner funnel. The solvent was evaporated in a rotary flash evaporator. After cooling at room temperature the crude extracts were stored at -4^{0}C. Crude extracts were diluted with methanol (1mg/ml) for further investigation.

Preliminary phytochemical screening

Preliminary phytochemical screening was performed to identify phytochemicals present in plant extract using standard procedures[17,18].

Thin Layer Chromatography (TLC)

The methanolic fractions were further separated by column chromatography on silica gel. The fractions obtained were analyzed by TLC. Analytical TLC plates were prepared by pouring silica gel G and GF slurry on the glass plates. The plates were allowed to dry in air for 30 minutes and then kept in oven at 110^{o}C for 30 minutes. The plates were placed in the developing jar with the mobile phase Toluene: Ethyl acetate (7:1). The chromatograms were observed in UV/VIS. The spots were identified and R_f values were calculated.

Spot visualization

Concentrated H_2SO_4 and Ehrlich reagent were used as spraying reagent. TLC plates were heated at 100^{0}C after spraying the reagent. Pinkish-violet spots of saponin were observed under UV/VIS.

Screening for Saponin

Froth test: Methanolic extract (0.5g) was dissolved in 10 ml distilled water in a test tube and was shaked vigorously for 30 seconds. The tube was allowed to stand in vertical position for 30 minutes. If froth was observed above the surface of liquid after 30 min the sample confirms the presence of Saponin[19].

Collection of the active compound

Spots of Saponin on the preparative silica gel plate were scratched with the help of clean and dry spatula and collected in air tight containers. These active compounds were further dissolved in methanol (1mg/ml) and were used for determination of antimicrobial effect.

HPTLC

The phytochemical screening was also done by high performance thin layer chromatography (HPTLC) using Camag-TLC scanner 3, winCATS software (Camag). For HPTLC, methanol extract was used and these studies were carried out on pre-coated aluminium fluorescent plates (E. Merck) were used. After development plate was scanned at 254 and 366nm[20]

Determination of antimicrobial activity

Antimicrobial activity of the active compound was tested by using disc diffusion method. Zone of inhibition (mm) was measured and mean values were tabulated.

Microorganisms

Staphylococcus aureus, Escherichia coli, Enterococcus fecalis, Pseudomonas aeruginosa and Proteus vulgaris were used to test antimicrobial activity. The bacterial cultures were obtained from Department of Microbiology, SGB Amravati University, Amravati (M.S.).

Antibacterial assay

Antimicrobial activity of the active compound extract (obtained from TLC) against crude extract (obtained from Soxhlet extraction) were determined by disc diffusion method. Three or four isolated colonies were inoculated in nutrient broth (2ml) and incubated at 37^{o}C for 12-14h till the growth in the broth is equivalent to 0.5 Mac-Farland standards. The standard inoculums were spread on the Mueller Hinton Agar plates using sterile cotton swabs. Sterile discs 6-mm diameter were impregnated with the different extracts of the *Chlorophytum* and were placed on the cultured plates. The plates were incubated at 37^{o}C for 24h. After overnight incubation the plates were observed and antibacterial activity was assessed. The experiments were carried out in triplicate. The results (mean value n=3) were recorded by measuring the zone of growth inhibition (ZI) around the disc. The diameter of the zone of active compound extract was then compared with the diameter of zone of inhibition produced by crude extract.

Table 2: Preliminary Phytochemical screening of *Chlorophytum*

Phytochemicals	Test/reagent	*Chlorophytum kolhapurens*	*Chlorophytum baruchii*
Alkaloids	Dragendorff's test	++	++
	Wagner's test	++	++
	Mayer's test	++	++
	Hager's test	++	++
Carbohydrates	Benedict's test	+	+
	Fehling's test	+	+
Tannins and Phenolic	Gelatin test	+	+
	Lead acetate test	+	+
Glycosides	Brontrager's test	-	-
Proteins and amino acids	Biuret test	+	+
	Ninhydrin test	+	+
Saponin	Froth test	++	++
	Haemolysis test	++	++
Steroids	Libermann-Buchard test	-	+
	Salkowski's test	-	+
Flavonoids	Shinoda test	-	-

Key: ++ = high concentration; + = low concentration; - = absent

Figure 3a: *Proteus vulgaris*

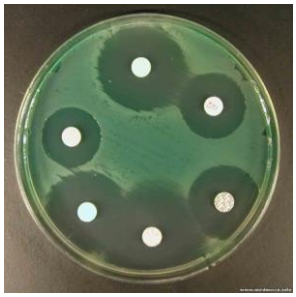

Figure 3b: *Pseudomonas aeruginosa*

Fig.3c: *Enterococcus faecalis*

Figure 3d: *Staphylococcus aureus*

Figure 3: Antimicrobial activity of *Chlorophytum*

Table 3: R_f Value of *Chlorophytum kolhapurens* and *Chlorophytum baruchii*

S. No.	Name	Rf Value
1	Standard Saponin	0.9
2	*Chlorophytum kolhapurens*	0.8
3	*Chlorophytum baruchii*	0.7

RESULTS

Physical properties of tuber powder of *Chlorophytum* species were analyzed. It was observed as whitish in color with characteristic taste (Table 1). The qualitative study carried out on the methanol extract of *Chlorophytum* species revealed the presence of medicinally active constituents such as alkaloids, carbohydrates, proteins, amino acids, saponins and phenols. Table 2 shows the various phytochemicals present in *Chlorophytum kolhapurens* and *Chlorophytum baruchii*. Table 3 shows the thin layer chromatograms (TLC) of phytochemicals separated from methanol extract of *Chlorophytum kolhapurens* and *Chlorophytum baruchii*. Solvent system Toluene: Ethylacetate (7:1) shows good separation and pinkish spots of saponins were observed with R_f values as 0.8 and 0.7. The HPTLC analysis showed saponins of *Chlorophytum* species gave yellow bands in visible light and blue bands after derivatization (figure 2). Table 4 shows the antimicrobial activity of *Chlorophytum* species (figure 3).

TLC

The solvent system selected for TLC of *Chlorophytum* was Toluene: Ethylacetate (7:1). The R_f value was recorded for each well resolved band.

HPTLC

The HPTLC analysis showed saponin extracted from tubers of *Chlorophytum kolhapurens* and *Chlorophytum baruchii* gave light yellow bands in visible light and blue bands after derivatization in fluorescence light. The plates were scanned at 254 and 366nm. *Chlorophytum borivilianum* was used as reference. From the graph and table values *Chlorophytum baruchii* showed maximum area 46.33% at 366 nm after derivatization (figure 2 a, b).

Antimicrobial activity

Antimicrobial activity on Gram positive and Gram negative bacteria was performed by disc diffusion method (Table 4 and Figure 3). *Chlorophytum* showed significant inhibitory activity against all tested pathogenic bacterial cultures. Crude extract of *Chlorophytum kolhapurens* was found to be most effective against *Proteus vulgaris* and less active against *Staphylococcus aureus. Chlorophytum baruchii* was found to be most effective against *Escherichia coli* and less active against *Staphylococcus aureus.* Whereas the active compound extract of *Chlorophytum kolhapurens* was found to be most effective against *Enterococcus faecalis* and less active against *Staphylococcus aureus. Chlorophytum baruchii* was found to be most effective against *Escherichia coli* and less active against *Enterococcus faecalis*. It was found that *Proteus vulgaris* and *Escherichia coli* were more sensitive as *C. kolhapurens* and *C.baruchii* showed 16mm and 15mm of inhibitory zones respectively.

DISCUSSION

The beneficial medicinal effects of plant materials typically result from the secondary products present in the plant, usually not attributed to a single compound but a combination of the metabolites. The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct[22]. Plant essential oils and extracts have been used for many thousands of years, in food preservation, pharmaceuticals, alternative medicine and natural therapies. Therefore, it is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare. Plants extracts are potential sources of novel antimicrobial compounds especially against bacterial pathogens. Studies showed that plant extracts inhibit bacterial growth but effectiveness varies. The antimicrobial activity of many plant extracts has been previously reviewed and classified as strong, medium or weak[23]. Antibiotics provide the main basis for the therapy of microbial infections. However, the high genetic variability of microorganisms enables them to rapidly evade the action of antibiotics by developing antibiotic resistance[24]. Also intensive use of antibiotics often resulted in the development of resistant strains[25], which create problems in treatment of infectious diseases, furthermore antibiotics are also sometimes attributed with side effects[26] whereas there are some advantages of using antimicrobial compounds of medicinal plants such as often fewer side effects, better patient tolerance,

Table 4: Antimicrobial activity of *Chlorophytum* by Disc diffusion method

SNo	Microorganisms	Zone of inhibition (mm) Mean±SD			
		C.kolhapurens (Crude)	*C.kolhapurens* (Active cpd.)	*C. baruchii* (Crude)	*C. baruchii* (Active cpd.)
1	*Escherichia coli*	11±1.7	12±1.6	15±2.4	14±2.2
2	*Enterococcus faecalis*	10±1.4	15±2.4	11±1.7	9±1.4
3	*Proteus vulgaris*	16±2.4	11±1.7	10±1.4	10±1.4
4	*Pseudomonas aeruginosa*	10±1.4	10±1.4	14±2.2	11±1.7
5	*Staphylococcus aureus*	9±1.4	8±0.9	9±1.4	12±1.6

relatively less expensive, acceptance due to long history of use and being renewable in nature[27]. Thus, there has been continuing search for new and more potent antibiotics[28]. In the present investigation the methanol extract (crude) and the active compound extract of *Cholorophytum* was evaluated for its antibacterial potential against pathogenic microorganisms. In classifying the antibacterial activity as Gram-positive or Gram-negative, it would generally be expected that a much greater number would be active against Gram-positive than Gram-negative bacteria[29], however, in our study both Gram-positive and Gram-negative bacteria are found to be sensitive to the plant extracts. *Cholorophytum* showed strong antimicrobial activity which certainly indicates that *Cholorophytum* contain higher concentration of active antimicrobial agents which is revealed by phytochemical analysis, TLC and HPTLC reports. The crude extracts of both species of *Cholorophytum* showed remarkable antimicrobial activity than active compound extracts. Variation in antimicrobial potential within species is due to intrinsic properties of the species. These observations will stimulate further research of phytochemical constituents of *Cholorophytum* species for clinical applications.

CONCLUSIONS

It was concluded that the methanolic extract of *Chlorophytum* species contains medicinally active constituents such as alkaloids, carbohydrates, proteins, amino acids, saponins and phenols. In was also concluded that both, *Chlorophytum kolhapurens* and *Chlorophytum baruchii* showed significant antimicrobial activity against pathogenic bacterial isolates indicating their possible use as antimicrobial agents. The obtained results are considered significant for isolation of compounds. Further study is required to isolate the compounds responsible for antimicrobial activity.

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