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RESEARCH ARTICLE

Phytochemical screening of selected Xerophytes of Ramdurg region of Belgavi District, Karnataka State

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ABSTRACT

Phytochemical screening tests were performed onselected xerophytes namely*Euphorbia milii* and *Agaveamericana* from Ramdurg region of Belgavi District, Karnataka State. Local vaidyas use *Euphorbia milii*to treat sprains and *Agave americana*to treat kidney stones and cleansing of blood. The phytochemical screening revealed the presence of terpenoids, flavonoids, tannins and saponins in aqueous and ethanolic extracts of the two species. The spectral studies of the crude extracts revealed several biologically active functional groups with promising antimicrobial activities.

Key words: Phytochemicals, *Euphorbia milii*, *Agave americana*, antimicrobial activity.

INTRODUCTION

The search for new drugs from plants and animals has a long history and dates back to 4000-5000 B.C. Isolation and characterization of pharmacologically active compounds from natural sources is an endless human endeavour. Phytochemicals exhibiting pharmacological activities have the potential to ease the growing demand for newer potent drugs.Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity (Samuelsson, G., 2004). According to the World Health Organization (WHO), nearly 20,000 medicinal plants exist in 91 countries including 12 mega biodiversity countries. More than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The use of herbal medicines in India represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Duraipandiyan *et al*., 2006).

Synthetic drugs have been used extensively for treatment of various ailments due to their quick effect. Increased instances of drug resistance and wide array of side effects of the synthetic drugs have forced us to look back at nature and natural ways to counter this problem.

Ramdurg region in Belgaum district, Karnatkastate is a home for several medicinal plants, many of which are xerophytic in nature. Traditional knowledge of Local Vaidyas about medicinal plants and their importance in local health carepractices is well known in this part of the country. However there is lack of information and documentation ofapplication of different medicinal plants. Local vaidyas use crude extracts of *Euphorbia milii*to treat sprains and *Agave americana*to treat kidney stones and cleansing of blood. Hence the present studywas undertaken in order to screen the Phytochemicals from *Euphorbia milii*and *Agave americana*

The genus *Euphorbia* is one of the largest genus ofmedicinal plants widely distributed in most part of theRamdurg. The plants are characterized by thepresence of milky latex which is more or less toxic. The latex is used to treat sprains.

The genus *Agave* is a very common perennial plant found in waste lands (Chetty *et al*., 2008). Literature attributes Agave to have chemical constituents like flavone glycoside, sterol glycosides etc.(Khare,2004) and is reported as laxative, emmenagogue, scurvy, aphrodisiac etc in folklore claims (Farooq, 2005)

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MATERIALS AND METHODS

Plant Material:

*Euphorbiamilii*and *Agave americana*were collected from the wild in the month of October, 2013. The plants were identified using standard keys and descriptions. The plants were compared with voucher specimens deposited at Department of Botany, JSS College, Dharwad. The voucher specimens of the collected plant materials were deposited at the Herbarium of the same department.

Extraction:

The Plant materials wereshade dried at roomtemperature for 10 days. The shade dried plant materials were crushed to make fine powder. The powderedmaterials (10g each) were soaked in 25ml of ethanol for5 days and then subjected to repeated extraction with 25×3ml until the extractant was colorless. The extractsobtained were then concentrated under reduced pressureusing rotary evaporator at temperature below 55°C. An equal quantity of plant materials were soaked in 50 ml distilled water for 5 days and finally the volume was reduced to 25 ml under reduced pressure.

Phytochemical screening:

Chemical screening was carried out on the aqueous and ethanolextracts by using standard procedure to detect theconstituents as described by Sofowora, Trease and Evans and Harborne.

Alkaloids:

About 0.2g of each extracts was warmed with2% $H_2SO_4$for two minutes. It was filtered and a few dropsof Dragendrof's reagent were added. Orange redprecipitate indicated the presence of alkaloids.

Tannins:

A small quantity of each extract was mixed withwater, heated on water bath and filtered. A few drops offerric chloride solution were added to the filtrate. A darkgreen coloration indicated the presence of tannins.

Anthraquinones:

About 0.5 g of each extract was boiledwith 10% HCl for a few minutes on water bath. It wasfiltered and allowed to cool. Equal volume of $CHCl_3$ wasadded to the filtrate. Few drops of 10% ammoniawas added to the mixture and heated. Formation of rose-pink color indicated the presence ofanthraquinones.

Glycosides:

Each extract was hydrolyzed with 2M HCland neutralized with 10% NaOH solution. A few drops ofFehling solution A and B were added. Red precipitateindicated the presence of glycosides.

Reducing Sugars:

The extracts were shaken withdistilled water and filtered. The filtrate was boiled with afew drops of Fehling's solution (A and B) for a fewminutes. An orange red precipitate indicated the presenceof reducing sugars.

Saponins:

0.2g extract was shaken with 5ml of distilledwater and then heated to boil. Frothing showed the presence ofsaponins.

Flavonoides:

0.2g extract was dissolved in diluted10%NaOH and 2M HCl was added. A yellow solutionthat turns colorless indicated the presence offlavonoides.

Phlobatanins:

0.5 g extract was dissolved in distilled waterand filtered. The filtrate was boiled with 2M HCl solution.Red precipitate showed the presence of Phlobatanins.

Steroids:

2 ml of acetic anhydride was added to 0.5g ofeach extract and then added 2 ml of H_2S0_4. The colorchanged from violet to blue or green or red whichindicated the presence of steroids.

Terpenoids (Salkowshki Test):

0.2g of the extract wasmixed with 2 ml of chloroform ($CHCl_3$) and concentrated6M H_2SO_4 (3ml) was carefully added forming a layer.A reddish brown coloration of the interface indicated thepresence of terpenoids.

IR spectra:

IR spectra were recorded on a Specord 71 IR spectrophotometer as potassium bromidediscs.

Antimicrobial Assay:

Test Microorganisms:

Aspergillus niger was used as the fungal test organism and *Escherichia coli* ATCC 25922 was used as the bacterial test organism. The pure microbial strains were obtained from the Department of Microbiology, University of Agricultural Sciences, Dharwad. The bacterial strains were cultured overnight at 37 °C in nutrient agar while fungal strains were cultured overnight at 28 °C using potato dextrose agar.

Disc Diffusion Method:

Antimicrobial activity of the ethanolic and aqueous extracts of the *Euphorbia milii*and *Agave americana*was determined by using the disc diffusion method of National Committee for Clinical Laboratory Standards. Wayne, PA, USA, 1997. All samples (dry residue) were dissolved in 10% sterile DMSO. The discs (6 mm diameter) were impregnated with 20 mg/mL extract (100 µL/disc) placed aseptically on the inoculated agar. Discs injected with 100 µL of ethanol served as negative controls, rifampcin (100 µL/disc) and fluconazole (100 µL/disc) were used as positive reference for bacteria and fungi, respectively. The petri dishes were incubated at 37 ± 0.1 °C for 20–24 h and 28 ± 0.3 °C for 40–48 h for bacteria and fungi, respectively. At the end of period, the inhibition zones formed on the media were measured. The positive antimicrobial activity was read based on growth inhibition zone.

ResazurinMicrotitre-Plate Assay

The minimum inhibitory concentration (MIC) of the plant extract/fractions was evaluated by a modified resazurinmicrotitre-plate assay reported by Sarker and co-workers with some modifications. Briefly, a volume of 100 µL of each extract and fractions solution in 10% dimethyl sulfoxide (DMSO, *v*/*v*) was transferred into the first row of the 96 well plates. To all other wells, 50 µL of nutrient broth and Muller Hinton broth for bacteria and fungi respectively were added. Two-fold serial dilutions were performed such that each well had 50 µL of the test material in serially descending concentrations. To each well, 10 µL of resazurin indicator solution (prepared by dissolving 270 mg resazurin tablet in 40 mL of sterile distilled water) were added. Finally, 10 µL of bacterial/fungal suspension were added to each well. Each plate was wrapped loosely with aluminum foil. Each plate had a set of controls: a column with broad spectrum antibiotics as positive control, a column with all solutions with the exception of the test samples, a column with all solutions with the exception of the bacterial/fungal solution adding 10 µL of broths instead and a column with respective solvents as a negative control. The plates were incubated at 37 ± 0.1 °C for 20–24 h and 28 ± 0.3 °C for 40–48 h for bacteria and fungi, respectively The absorbance was measured at 620 nm for fungus and at 500 nm for bacteria. The color change was then assessed visually. The growth was indicated by color changes from purple to pink or colorless. The lowest concentration at which color change appeared was taken as the MIC value.

RESULTS

Phytochemical screening on the crude ethanolic and aqueous extracts of the *Euphorbia milii*and *Agaveamericana*were undertaken. The qualitative presence of various secondary metabolites such as alkaloids, steroids, terpenoids, flavonoides and tannins, etc. was tabulated (**Table 1 & 2**). Infra red spectra of the ethanolic extracts of the *Euphorbia milii*and *Agave americana*were recorded and tabulated (**Table 3**). The ethanolic extracts of the *Euphorbia milii*and *Agave americana*were subjected to the antimicrobial assay and minimum inhibitory concentration (MIC) determination by Disc Diffusion Method and ResazurinMicrotitre-Plate Assay respectively.(**Table 4 & 5**)

Table: 1 Phytochemical screening of the crude extracts of *Euphorbia*

Chemical Components	Ethanol extract	Aqueous extract
Alkaloids	+	-
Steroids	-	-
Terpenoids	+	-
Flavonoids	+	-
Anthraquinones	-	-
Tannins	+	+
Phlobatanins	-	-
Saponins	-	-
Glycoside	-	+
Reducing sugars	-	+

+ denotes presence; – denotes absence

Table 2: Phytochemical screening of the crude extracts of *Agave*

Chemical Components	Ethanolic extract	Aqueous extract
Reducing sugar	+	+
Alkaloids	-	-
Tannins	-	-
Steroids	+	-
Cardiac glycosides	-	-
Saponin	+	-
Glycosides	-	+
Flavonoides	-	-

+ denotes presence; – denotes absence

Table 3: IR Spectroscopic data of the crude ethanolic extract of Euphorbia and Agave

Functional groups	Euphorbiamilli	Agaveamericana
	Region cm^{-1}	
CH	*2916.37*	*2916.37*
NO_2	*1558.48,1506*	*1506.59*
C-N	*1361.74*	1372.89
Ar-O	*1242.16*	1246.22
C-O-	*1068.56*	1075.26
R-O-	*1033.85*	1049.86
C=O	*1716.65*	1716.65
C=C	*1608.62*	1616.35
OH	*3346.50*	*3346.50*

Table: 4 Antimicrobial activity of Crude Ethanolic extract of *Euphorbiamilli* and *Agaveamericana*

Tested Microorganism	Diameter of Inhibition Zone, (mm)		Standard Drugs	
	Euphorbia ethanolic extract	Agave ethanolic extract	Rifampcin	Fluconazole
E. coli	20.0 ± 1.22	22.2 ± 0.82	21.5 ± 2.06	Not Detected

A. niger	22 ± 0.707	19.2 ± 1.47	Not Detected	18.5 ± 1.11

Table 5: Minimum Inhibitory Concentration (MIC) mg/mL.of Crude Ethanolic extract of *Euphor biamilli* and *Agave americana*

Tested Microorganism	Euphorbia ethanolic extract	Agave ethanolic extract	Rifampcin	Fluconazole
E. coli	65 ± 0.75	68 ± 0.87	62.1 ± 0.45	Not Detected
A. niger	92 ± 0.75	104 ± 2.85	Not Detected	98.2 ± 0.55

DISCUSSION

In the present work two xerophytes namely,*Euphorbia milii*and *Agave americana*were selected for phytochemical screening followed by spectral analysis and antimicrobial assay. The ethanolic extracts Euphorbia exhibited the presence of alkaloids, terpenoids, flavanoids and tannins while the aqueous extract shows the presence of tannins, reducing sugars and tannins. Similarly, Agave exhibited the presence of reducing sugars, steroids and saponins in the ethanolic extract while only reducing sugars and glycosides in the aqueous extract. The IR spectral analysis of the ethanolic extracts of the Euphorbia and Agave exhibited the presence of aromatic group (1242.16 cm^{-1} ,1246 cm^{-1}) followed by –NO_2 and –OH groups which indicated their antimicrobial activity. Further in the antimicrobial assay also the results indicate that the Euphorbia and Agave plants can be explored for antibacterial as well as antifungal agents. In fact the minimum inhibitory concentrations (MIC) of the tested samples are similar to the control samples (rifampicin and Flucanozole). This indicates that the ethanolic extracts of both Euphorbia and Agave plants are useful sources of pharmacologically viable drugs.

CONCLUSION

Data obtained in the present study points to the considerable antimicrobial activities possessed by the *Euphorbia milii* and *Agave Americana* plants. The presence of biologically important phytochemicals in the plant extracts may contribute to their medicinal value and potential sources for useful drugs. The investigated plants may be processed for pharmaceutical and natural therapies for the treatment of ailments in humans.

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