Research Article

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Preliminary phytochemical analysis, antibacterial activity and GC-MS analysis of *Kalanchoe floribunda* Wight&Arn.

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ABSTRACT

Traditional medicine also known as indigenous or folk medicine comprises medical knowledge systems that developed generations with in various societies before the era of modern medicine. In the present study deals with the phytochemical, antibacterial activity and GC-MS analysis of the medicinal plant *Kalanchoe floribunda* Wight & Arn. Qualitative phytochemical analysis of these plants confirms the presence of various secondary metabolites like alkaloids, phenols, proteins, saponins, sterols. Antibacterial activity of *Kalanchoe floribunda* leaf extracts (Ethyl acetate, Ethanol and Distilled Water) on pathogenic bacterial strains (*Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella oxytoca*, *Proteus mirabilis* and *Salmonella typhi*) was tested by agar well diffusion method. Ethyl acetate leaves were also should maximum inhibition on *Enterobacter aerogenes* (10mm) and *Klebsiella oxytoca* (10mm). Seven compounds were identified in the ethyl acetate extract of *Kalanchoe floribunda* by Gas Chromatography Mass Spectrometry (GC-MS) analysis.

Key words: *Kalanchoe floribunda* , phytochemical screening, antibacterial activity, solvents, bacterial strains, GC-MS analysis.

INTRODUCTION

Medicinal plants have been used by human being since ages in traditional medicine due to their therapeutic potential and the search on medicinal plants have led to the discovery of novel drug candidates used against diverse diseases. According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs (Pierangeli *et al.,* 2009). Higher plants as sources of bioactive compounds continue to play a dominant role in the maintenance of human health. Reports available on green plants represent a reservoir of effective chemotherapeutants, these are non-phytotoxic, more systemic and easily biodegradable (Vyas, 1999; Kaushik *et al.,* 2002). Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties (De Fatima *et al.*, 2006).

Antibacterial constituents of medicinal plants and their use for the treatment of microbial infections as possible alternatives to synthetic drugs to which many infectious microorganisms have become resistant seem to be very much promising (Bari *et al.,* 2010). Over the past 20 years, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents. Different extracts from medicinal plants were tested and some natural products were approved as new antibacterial drugs (Chehregani *et al.,* 2007). The medicinal value of plants lies in some chemical substances that produce a definite physiological action the human body (Ahsan *et al.*, 2009; Balakumar *et al.,* 2011; Rajan *et al.,* 2011; Anpin Raja *et al.*, 2011).

Antibiotics are sometimes associated with side effects whereas there are some advantages of using antimicrobial compounds of medicinal plants. The later has fever side effects, better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature (Reddy *et al.,* 2010).

A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be of great value in disclosing new sources of economic phytocompounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies (Milne *et al.,* 1993). Hence a thorough validation of the herbal drugs has emerged as a new branch of science emphasizing and prioritizing the standardization of the natural drugs and products because several of the phytochemicals have complementary and overlapping mechanism of action. Mass spectrometry, coupled with chromatographic separations such as Gas chromatography (GC/MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants. In recent years GC-MS studies

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have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non polar components and volatile essential oil, fatty acids, lipids and alkaloids. Gas chromatography has a very wide field of applications. But, its first and main area of use is in the separation and analysis of multi component mixtures such as essential oils, hydrocarbon and solvents.

MATERIALS AND METHODS

Plant Collection

The leaves of *Kalanchoe floribunda* Wight & Arn. was collected from Poondi garden, Thanjavur (Dt.) brought into the laboratory for further processes.

Sterilization of Plant Materials

The disease fresh leaves were selected for this investigation. About 2gm of leaves was taken for each solvent extract including aqueous. Then surface sterilized with 0.1% mercuric chloride or alcohol for few seconds. Again the plant materials were washed thoroughly with distilled water (Three times).

Preparation of Plant Extract

Two grams of sterilized plant leaves were kept in the 10ml organic solvent such as ethyl acetate, ethanol and distilled water. Then there are ground with the help of mortar and pestle. The ground plant materials were subjected to centrifugation for further antibacterial screening purpose.

Selection of Microorganisms

Totally five human pathogenic bacterial cultures were selected for the present investigation. Among them, five bacterial strains such as *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella oxytoca*, *Proteus mirabilis*, and *Salmonella typhi*. The bacterial cultures were originally obtained from Microbial Germ Plasm Culture Collection Unit (MGPCCU), Sri Gowri Biotech Research Academy, Thanjavur and used for present investigation.

Preparation of Microbial Inoculums

The young microbial cultures were prepared and used during the research period. The Nutrient Broth (NB) was prepared and poured into several tubes. Then these tubes were sterilized. The pure microbial cultures were collected from the institute and inoculated in the tubes by using inoculation needles or loops. After these tubes were incubated (37°C for 24-48 hrs for bacteria). After incubation the cultures were used for the experiments.

Preparation of Nutrient Agar Medium

1000ml of Nutrient agar medium are prepared pH was adjusted to 6.8, using a pH meter by the addition of either acid or alkali. The medium are sterilized by using autoclave of 121°C for 15lbs pressure for 15 minutes and allowed to cool.

Antibacterial Activity (Agar well diffusion method)

The antibacterial activities of the seeds were tested against the selected bacterial strains. The 20ml of sterilized Nutrient agar medium was poured into each sterile petriplates and allowed to solidify. The test bacterial cultures were evenly spread over the appropriate media by using a sterile cotton swab. Then a well of 0.5mm are made in the medium by using a sterile cork borer, 200µl of each ethyl acetate, ethanol and distilled water leaves extracts were transferred into separate wells. After these plates were incubated at 37°C for 24-48 hours. After incubation period, the results were observed and measure the diameter of inhibition zone around the each well.

Preliminary phytochemical screening of leaves extracts of *Kalanchoe floribunda* Wight & Arn.

All the leaves extracts such as ethyl acetate, ethanol and distilled water extract of *Kalanchoe floribunda* Wight & Arn. were subjected to preliminary phytochemical analysis to identify the nature of phytochemical constituents present in them (Brindha *et al*., 1982; Harborne, 1998).

Alkaloids

Two ml of test solution are taken with 2N HCl. Aqueous layer formed was decanted and then added with one or a few drops of Mayer's reagent. Formation of white precipitate or turbidity formed indicates the presence of alkaloids.

Sterols

Two ml of test solution and minimum quantity of chloroform are added with 3-4 drops of acetic anhydride and one drop of concentrated H_2SO_4. Formation of purple color changes into green color that indicates the presence of steroids.

Phenols

Two ml of test solution in alcohol are added with one drop of neutral ferric chloride (5%) solution. Formation of intense blue color indicates the presence of phenols.

Saponins

Two ml of test solution are added with H_2O and shaked. Formation of foamy lather indicates the presence of saponins.

Proteins

Picric acid is added with the test solution. Formation of yellow color indicates the presence of protein.

Gas Chromatography and Mass spectrometry (GC-MS) analysis of ethyl acetate leaves extract in *Kalanchoe floribunda* Wight & Arn.

The GC-MS analysis was carried out using a Clarus 500 Perkin – elmer (Auto system XL) Gas Chromatograph equipped and coupled to a mass detecter Turbo mass gold – perkin Elmer Turbomass 5.1 spectrometer with an Elite – 1(100% Dimethyl poly siloxane), 30m x 0.25 mm ID x 1µm of capillary column. The instrument was set to an initial temperature of 110°C, and maintained at this temperature for 2 min. At the end of this period the oven temperature was rose up to 280°C, at the rate of an increase of 5°C/min, and maintained for 9 min. Injection port temperature was ensured at 250°C and Helium flow rate at one ml/min. The ionization voltage was 70eV.The samples were injected in

split mode as 10:1.Mass spectral scan range was set as 45-450 (m/z). Using computer searches on a NIST Ver.2.1 MS data library and comparing the spectrum obtained through GC-MS componends present in the crude sample were identified.

RESULTS

In the present investigation the preliminary phytochemical analysis and antibacterial activity of ethyl acetate, ethanol and distilled water extract of *Kalanchoe floribunda* Wight & Arn. was strains tested against five human pathogenic bacterial strains such as *Enterobacter aerogenes, Escherichia coli, Klebsiella oxytoca, Proteus mirabilis,* and *Salmonella typhi.*

Anti bacterial activity of leaf extract in *Kalanchoe floribunda* Wight & Arn.

Elaissi *et al*., (2012) reported that the different oils was evaluated by the paper-disc agar diffusion method against the two gram-negative model bacteria *Escherichia coli* and *Pseudomonas aeruginosa* and the two gram-positive bacteria *Staphylococcus aureus* and *Enterococcus faecalis*. In our study the ethyl acetate extract is exhibited zone of inhibition against, *Klebsiella oxytoca* (10mm) and *Enterobacter aerogenes* (10). Ethanol extract are should signification. Antibacterial activity against *Escherichia coli* (8mm) and *Proteus mirabilis* (8mm). Distilled water extract are should minimum zone of inhibition against *Enterobacter aerogenes, Proteus mirabilis* (5mm) and *Salmonella typhi* (5mm) (Table 1).

Table: 1 Antibacterial activity of leaf extract in *Kalanchoe floribunda* Wight & Arn.

Bacterial cultures	Solvent extracts (Zone of inhibition in mm)		
	Ethyl acetate	Ethanol	Distilled water
Enterobacter aerogenes	10	6	5
Escherichia coli	8	8	-
Klebsiella oxytoca	10	7	-
Proteus mirabilis	6	8	5
Salmonella typhi	8	7	5

Antibiotic sensitivity test on bacteria (Positive control)

Bahrami and Ali, (2010) investigated that the antibacterial assay, the essential oil (100µg/mL) showed comparable antibacterial activity of that of the positive control ampicillin (10µg/mL) against *Staphylococcus arueus.* This is the first report on the antibacterial activity of the essential oil of this species. However, other species of the genus *Scrophularia* have previously been shown to have antibacterial activity against *Staphylococcus aureus.* In the present study the antibiotic sensitivity test such as Erythromycin, Kanamycin, and Streptomycin were tested against bacterial strains (Table 2).

Table: 2 Antibiotic sensitivity tests on bacteria (Positive control)

Bacterial cultures	Standard antibiotics (Zone of inhibition in mm)		
	Erythromycin	Kanamycin	Streptomycin
Enterobacter aerogenes	-	11	13
Escherichia coli	5	8	3
Klebsiella oxytoca	9	11.5	10
Proteus mirabilis	10	15	15
Salmonella typhi	15	10	12

Effect of solvents on bacteria (Negative control)

The result of negative control experiments (solvents) showed known antibacterial activity (Table 3).

Table: 3 Effect of solvents on bacteria (Negative control)

Bacterial cultures	Solvent extracts (Zone of inhibition in mm)		
	Ethyl acetate	Ethanol	Distilled water
Enterobacter aerogenes	-	-	-
Escherichia coli	-	-	-
Klebsiella oxytoca	-	-	-
Proteus mirabilis	-	-	-
Salmonella typhi	-	-	-

Phytochemical analysis of leaf extract in *Kalanchoe floribunda* Wight & Arn.

Preliminary phytochemical analysis of the different solvent extracts such as ethyl acetate, ethanol, and distilled water of leaves in *Kalanchoe floribunda* Wight & Arn. indicates the presence of alkaloids, sterols, phenols, saponins, proteins (Table 4).

Table: 4 Preliminary phytochemical analysis of *Kalanchoe floribunda* Wight & Arn.

Phytocompounds	Ethyl acetate	Ethanol	Distilled Water
Alkaloids	+	+	-
Sterols	+	+	-
Phenols	+	+	+
Saponins	+	-	-
Proteins	+	+	+

+ Present, - Absent

Table :5 GC – MS analysis of leaf extract in *Kalanchoe floribunda* Wight&Arn.

RT	Name of the compounds	Molecular Formula	MW	Peak Area%
10.99	Dodecanoic acid	$C_{12}H_{24}O_2$	200	1.61
13.47	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	39.70
16.34	n-Hexadecadienoic acid	$C_{16}H_{32}O_2$	256	11.14
18.96	9,12-Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	280	5.45
21.26	Pregnanetrial	$C_{21}H_{36}O_3$	336	4.83
22.35	5-(1-Lsopropenyl-4,5 dimethylbicyclo(4.3.0) nonan-5-yl)-3-methyl-2-pentenol acetate	$C_{22}H_{36}O_2$	332	3.15

GC-MS analysis of ethyl acetate leaf extract in *Kalanchoe floribunda* Wight & Arn.

Rosy and Rosakutty, (2012) reported that the chemical composition of methanol wild plant and callus extracts of *C. xavierensis, C. quadrangularis* Var. *rotundus* and *C. vitiginea* were analyzed using GC-MS analysis. In the present study GC-MS analysis has also revealed the presents of six bioactive compounds in the ethyl acetate extract (Table 5and Fig 1). The bioactive compounds form the extract was identified with there retention time, molecular formula, molecular weight and concentration (%). The predominant compound present in ethyl acetate extract of *Kalanchoe floribunda* Wight & Arn. are Dodecanoic acid (1.61%), Tetradecanoic acid (39.70%), n-

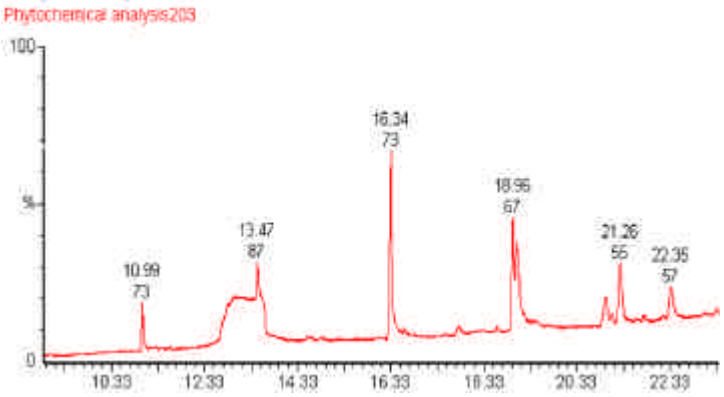

Fig: 1 GC – MS analysis of leaf extract in *Kalanchoe floribunda* Wight&Arn.

Hexadecadienoic acid (11.14%), 9,12-Octadecadienoic acid (Z,Z) (5.45%), Pregnanetrial (4.83%). The remaining compounds of least level and they are 5-(1-Lsopropenyl-4,5dimethylbicyclo(4.3.0) nonan-5-yl)-3-methyl-2-pentenol acetate (3.15%).

CONCLUSION

A scientific and systematic investigation with regard to the various biological activities of this plant is lacking. Results of the present work indicate that the plant species assayed possess antimicrobial properties. This explains the use of these plants in folk medicine for the treatment of various diseases whose symptoms might involve bacterial and fungal infections and underline the importance of the ethnobotanical approach for the selection of plants in the discovery of new bioactive compounds. Further phytochemical research is needed to identify the active principles responsible for the antibacterial effects of some of these medicinal plants.

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