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RESEARCH ARTICLE

Isolation, identification and characterization of the biologically active compound in ethyl acetate extract of *Sansevieria zeylanica* and evaluation of its antimicrobial effect against *Klebsiella pneumoniae*

Vysakh G[1], Sakthi Babu R L[1*]

P. G Department of Zoology & Research Centre, Mahatma Gandhi College, Thiruvananthapuram

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Corresponding Author

Sakthi Babu RL

Abstract

The present study was aimed to isolate and identify the active compound in ethyl acetate extract of *Sansevieria zeylanica* and evaluation of its antimicrobial effect against *Klebsiella pneumoniae*. Dried powdered plant material was used in this study, extracted with ethyl acetate using soxhlet apparatus. Column chromatography was used for the separation and purification of active compounds in ethyl acetate extract of *Sansevieria zeylanica*. FT IR spectrum, HNMR spectrum,13CNMR spectrum analysis were used for elucidating the structure of isolated compounds of *Sansevieria zeylanica*. The phytochemical analysis of the compound was done by using standard procedure. The anti microbial effect of the active compound was evaluated by disc diffusion method. Phytochemical analysis of compound isolated from ethyl acetate extract of *Sansevieria zeylanica* shows the presence of phenolic compounds. Analysed peaks of FT IR spectrum, HNMR spectrum,13CNMR strongly suggested that the experimental compound may be a phenyl-2-hydroxy decyl ketone. The antimicrobial assay of phenyl-2-hydroxy decyl ketone confirms the efficacy of the compound as anti bacterial agent and also used as drug for treating various disease associated to *Klebsiella pneumoniae*.

1. INTRODUCTION

Klebsiella pneumoniae is a member of the *Klebsiella* genus of Enterobacteriaceae and belongs to the normal flora of the human mouth and intestine (Ryan et al., 2004). Of the pathogenic *Klebsiella* species, *K. pneumoniae* is the most prevalent and clinically important. Infections with *K. pneumoniae* are usually hospital-acquired and occur primarily in patients with impaired host defences. In addition to pneumonia, *Klebsiella* can also cause infections in the urinary tract, lower biliary tract, and surgical wound sites. The range of clinical diseases includes pneumonia, thrombophlebitis, urinary tract infects (UTI), cholecystitis, diarrhea, upper respiratory tract infection, wound infection, osteomyelitis, meningitis, and bacteremia and septicemia. The high rate of nosocomial *Klebsiella* colonization appears to be associated with the use of antibiotics rather than with factors connected with delivery of care in the hospital (Pollack et al., 1972 and Rose et al., 1968). Sepsis and septic shock can follow entry of the bacteria into the blood. *Klebsiella* ranks second to E. coli for urinary tract infections in older persons. It is also an opportunistic pathogen for patients with chronic pulmonary disease, enteric pathogenicity, nasal mucosa atrophy, and rhinoscleroma.

Now a day, over and incorrect use of antibiotics was the major reason for the emergence of multi drug resistant strains. Some bacteria develop genes for drug resistance in plasmids; they are able to spread drug resistance to other strains. Overuse of antibiotics leads to major side effects such as kidney failure, nephro toxicity etc. For Centuries plants have been used throughout the world as drugs and remedies for various diseases (UNESCO, 1996).

Sansevieria zeylanica (vishappola) belongs to the family Agavaceae. Two to six long leaves are present in each plant. Leaves are broad, thick, fleshy, dark green with numerous very conspicuous, light or yellowish green, irregularly confined transverse bands, in the normal form with a narrow dark green margin. The medicinal uses of Sansevieria species include treatment of abdominal pains, ear ache, diarrhoea and haemorrhoids (Van Wyk et al., 1997).The warm juice of this plant leaves are dropped in to the ear as a treatment for earache. The juices of fresh leaves are used to treat pharyngitis and hoarseness. A warm decoction of the leaves is applied to itchy skin. The leaf sap is applied directly to infected sores, cuts and grazes. It is also used to treat fungal and scabies infection (Olivia Case, 2005 and Traditional Medicine Database, 2002).

Hence, the present study, an attempt was made for extraction, isolation, characterization and identification of phytochemicals from the ethyl acetate extract of selected medicinal plant *Sansevieria zeylanica* against *Klebsiella pneumoniae* and thereby providing a ray of hope to find alternative solutions for antibiotics to prevent the emergence of resistant strains of bacteria.

2. MATERIALS AND METHODS

2.1 Chemicals

The chemicals such as Hexane, Chloroform, Ethyl acetate, DMSO, Potassium Mercuric Iodide, Potassium Bismuth Iodide, Picric acid, Benedicts reagent, Fehlings reagent, Ferric Chloride, Benzene, Ammonia, Chloroform, Acetic anhydride, Concentrated Sulphuric acid and other chemicals (analytical or equivalent grade of high purity) used for the experiment were purchased from SRL, Bombay.

2.2 Plant material

Sansevieria zeylanica was locally collected from Thiruvananthapuram district, Kerala, and botanically authenticated. The long leaves taken from *Sansevieria zeylanica*, were washed thoroughly 2 – 3 times with running water and dried in 40 – 60 ºC in hot air oven. The dried material was finely powdered and stored in air tight bottle and used for soxhlet extraction.

2.3 Isolation of ethyl acetate fraction

A quantity of 500 gm of hot air dried powdered plant material was extracted in 2.5 L of ethyl acetate using soxhlet. The soxhletion with ethyl acetate was done for one week to obtain extract. The final extract of *Sansevieria zeylanica (S. zeylanica)* collected was concentrated under rotary evaporator and preserved at 4ºC in air tight bottles for conducting the further experiments.

2.4 Isolation of active ingredients

The concentrated extract obtained from ethyl acetate, which is to be separated was first applied to the column at the top. When all the concentrated extract had been adsorbed on the top of the column, more hexane was added and the column was allowed to run. Some compounds of the mixture are adsorbed strongly, while others less strongly. The more strongly a substance is adsorbed, the more slowly it moves down the column. The elution was carried out using the mixture of hexane and chloroform (9:1 i.e. 90:10 Hexane : Chloroform). Each 50.0 ml fraction was separately collected for a period of 30 days. Primary analysis of the compound purity of this collected fractions were done by TLC. Identical fractions were pooled together (Bobbitt, 1963).

2.5 Identification of compound purity

Thin layer chromatography was performed to analyze each fraction. Glass plates were coated with slurry of TLC silica gel to about 0.2 mm thickness and allowed to dry in air. Dried TLC plates were activated at $ll0^{0}$ C and spotted with the eluted fraction using a small capillary tube. The plate was dipped in a tank containing suitable solvent. The choice of solvent depends on nature of substances to be separated and also the material on which separation is achieved. The solvent in this case was a mixture of hexane and chloroform. When the solvent front has reached sufficient heights it was removed. The plate was now taken out, dried and placed in an iodine chamber for the conformation of single compound in the isolated fraction. The eluted pooled fractions (9:1 of hexane, chloroform) were concentrated under rotary evaporator. The concentrated material was crystallized and re-crystallized using chloroform and preserved in the desiccator at 4ºC in air tight bottles for conducting the study.

2.6 Characterization of isolated compound

The eluted, purified and crystallized compound obtained from the ethyl acetate extract was subjected to spectral analysis for chemical characterization. In this study, ^{1}H NMR, ^{13}C NMR, FT IR was used for elucidating the structure of isolated compounds of *Sansevieria zeylanica*. FT IR Spectroscopy is mainly used for the determination of functional groups. IR spectrum is usually presented as a plot of percentage radiation of each wave length transmitted through the sample. TMS (Tetra Methyl Silane) signal is used to calibrate NMR spectra because it is inert, soluble in most organic solvents and volatile enough to be easily removed after the spectrum is obtained.

Usually a small amount of TMS is added directly to a sample before the sample is run. The difference in frequency between the sharp TMS peak and the absorption signals in another proton is called the chemical shift.

2.7 Estimation of Phytochemicals

The re-crystallized ingredient obtained from the eluted fractions, which is to be analyzed, was used as test material.

2.7.1 Test for alkaloids

Mayer's test

A fraction of extract was treated with Mayer's test reagent (1.36 g of mercuric chloride and 5g of potassium iodide in 100 ml of water) and observed for the formation of cream colored precipitate.

Wagner's test

A fraction of extract was treated with Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml water) and observed for the formation of reddish brown colour precipitate.

Dragendroff's test

To small quantity (10µg) of crystal 1.0ml of Dragendroff's reagent was added. Appearance of orange precipitate indicated the presence of alkaloids.

2.7.2 Test for Flavonoids

A small quantity (10 µg) of crystal is heated with 10 ml of ethyl acetate in boiling water for 3 minutes. The mixture is filtered differently and the filtrates are used for the following test.

Ammonium Test

The filtrate was shaken with 1 ml of dilute ammonia solution (1%). The layers were allowed to separate. A yellow coloration was observed at ammonia layer. This indicates the presence of the flavonoid.

Aluminum Chloride Test

The filtrates were shaken with 1 ml of 1% aluminum chloride solution and observed for light yellow color. It indicated the presence of flavonoid and diluted NaOH and HCl was added. A yellow solution that turns colorless indicated positive.

2.7.3 Test for Terpenoids

Salkowski Test

The 10 µg crystal was mixed with 2ml of chloroform and concentrate H2SO4 (3ml) is carefully added to form a layer. A reddish brown coloration of the interface is formed to show positive result of the presence of terpenoids.

2.7.4 Test for Phenol

Ferric Chloride Test

To 1ml of the extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green color indicates presence of phenols.

2.7.5 Test for Tannins

A small quantity (10 µg) of crystal was boiled with 5 ml of 45% solution ethanol for 5 minutes. Each of the mixture is cooled and filtered. The different filtrates were used to the following test:

Lead Sub Acetate Test

1ml of the different filtrate was added with three drops of lead sub acetate solution. A cream gelatinous precipitation indicates positive test for Tannins.

Ferric Chloride Test

1ml each of filtrate was diluted with distilled water and added with two drops of ferric chloride. A transient greenish to black color indicates the presence of Tannins.

2.7.6 Test for Steroids

2ml of acetic anhydride was added to 10 µg of crystals with 2ml of H2SO4. The color changed from violet to blue or green in some samples indicated the presence of steroids.

2.7.7 Test for Saponins

Frothing Test

A small quantity (10 µg) of crystals was diluted with 4 ml of distilled water. The mixture was shaken vigorously and then observed on standing for stable brake.

2.7.8 Test for Glycosides

5ml of diluted sulphuric acid was added in 10 µg crystals in a test tube and boiled for fifteen minutes in a water bath. It was then cooled and neutralized with 20% potassium hydroxides solution. A mixture of 10ml of equal

parts of Fehling's solution A and B were added and boiled for five minutes. A more dense red precipitate indicates the presence of glycosides.

2.7.9 Test for Reducing Sugar

A small fraction (10 µg) of crystals was added vigorously with 5ml of distilled water and filtered to the filtrates while equal volumes of Fehling's solution A and B added and were shaken vigorously. A brick red precipitation indicated positive.

2.7.10 Test for Carbohydrates

10 µg crystals were shaken vigorously with water and then filtered. To the aqueous filtrate was added few drops of Molisch's reagents. Followed by vigorous shaking again, concentrated H2SO4 (1ml) was carefully added to form a layer below the aqueous solution. A brown ring at the interface indicated the positive.

2.7.11 Test for Proteins

5ml of distilled water was added into the 10 ug crystals. This was left to stand for three hours and then was filtered. To 2ml portion of the filtrate was added 0.1ml Millon's reagent. It was shaken and kept for observation. A yellow precipitation indicates the positive.

2.8 Antimicrobial effect of isolated crystals on *Klebsiella pneumonia*

The isolated crystals were treated as study material and were made into a suspension using 10% dimethylsulphoxide (DMSO) in distilled water. The concentration of the material was made in 1mg/ml. The antibacterial activity of isolated crystals was studied by disc diffusion assay.

2.8.1 Preparation of test paper discs

The 6.0 mm filter paper discs were impregnated with 50 µl and 100 µl of isolated crystals for about 30 minutes. For standard antibiotics readymade discs were used as positive control and the disc impregnated with 50 µl and 100 µl of 10% DMSO alone used as negative control.

2.8.2 Experimental organism

Pure slant cultures of *Klebsiella pneumoniae* were collected from the Research laboratory of South Travancore Hindu College, Nagercoil, Kanyakumari District. The slant cultures were brought to the laboratory within a short time to avoid any possible contamination. It was stored at 4 ºC in refrigerator for future preparation of sub culture in nutrient agar.

2.8.3 Experimental design

All the *Klebsiella pneumoniae* inoculated plates were divided into five groups. (Each group contains four (4) *K. pneumoniae* inoculated plates respectively).

Group SI – received filter paper discs were impregnated with 50 µl of isolated crystals.
Group SII – received filter paper discs were impregnated with 100 µl of isolated crystals.
Group SIII – received disc impregnated with 50 µl of 10% DMSO alone.
Group SIV – received disc impregnated with 100 µl of 10% DMSO alone.
Group SV – standard antibiotics

2.8.4 Antibacterial Susceptibility Test

Muller Hinton agar was used as media for antibacterial susceptibility test. After the agar surface had dried, the appropriate sterilized filter paper discs were impregnated with 50 µl and 100 µl of isolated crystals for about 30 minutes were placed on the inoculated agar plates using sterile forceps. The discs were placed sufficiently distant from each other in order to avoid the mixing of inhibition zone. Then plates were incubated at 37 ºC for 24 hrs. After 24 hours of incubation the culture plates were observed to check whether the selected medicinal plants have any antibacterial activity against *Klebsiella pneumoniae*. The antimicrobial activity was expressed as IZD mm (Inhibition Zone Diameters) produced by isolated crystals and known antibiotics as standard against the *Klebsiella pneumoniae*.

2.8.5 Statistical Analysis

Standard deviations were tested with the data of zones of inhibition developed by filter paper discs were impregnated with 50 µl and 100 µl of isolated crystals from the ethyl acetate extract of *Sansevieria zeylanica* on *Klebsiella pneumoniae.* The Student t test was conducted with diameters of zones of inhibition of all treated groups ((Group S – SI & SII (Experimental) with SIII & SIV (Control groups) and Group S – SI & SII with SV (Positive Control). The results are expressed as mean + SE, and Paired Samples test (SPSS - 19 Computer package) was used to assess statistical significance.

3. RESULTS

3.1 Phytochemical analysis of active compound from *Sansevieria zeylanica*

The result of qualitative analysis of various phytochemicals present in isolated crystals from ethyl acetate extract of indigenous drug *Sansevieria zeylanica* is presented in Table 1. The various qualitative tests were conducted to determine the presence of alkaloid in isolated crystals showed its absence. The test materials exposed with ammonium test and ammonium chloride test indicated the absence of Flavonoids. Results of Salkowski test indicated absence of terpenoids. No creamy gelatinous precipitation and no color change appeared in the crystals isolated from ethyl acetate extract subjected to Lead Sub Acetate Test and ferric Chloride test respectively revealed the absence of tannins. Crystals subjected to ferric chloride test resulted the formation of bluish black colour indicated the presence of phenols. No colour change was noted in the sample treated with acetic anhydride and concentrated Sulphuric acid indicated the absence of steroids. Failure in the formation of foam was recorded in the sample revealed the absence of saponins. Various qualitative tests conducted to determine presence of glycosides, reducing sugar and carbohydrates in the purified crystals of selected medicinal plant showed their absence. The extract when treated with 0.1ml Millon's reagent did not produce characteristic yellow precipitation which indicated the absence of proteins. All the above mentioned secondary metabolites have a definite physiological action on the human body. The phytochemical screening revealed that isolated crystal is rich in phenols.

3.2 Analysis of FT IR spectrum

FT IR spectrum of this study showed absorption in the following wave numbers 702.97, 743.37, 1039.95, 1072.52, 1123.24, 1273.80, 1380.98, 1463.26, 1730.40, 2853.86 and 2922.89. The peak at 702 and 743.37 cm-1 is due to aromatic ring and that at 1730.40 cm-l is due to double bond of carboxy (C = O) vibrations of ketone. The peaks at 2853.86 and 2922.89 are due to C – H vibrations of a long chain (figure 1).

3.3 Analysis of ^{1}H NMR spectrum

Our study revealed that the observed ^{1}H NMR spectrum showed absorption in the following wave numbers 4.205, 4.220, 4.234, 7.512, 7.520, 7.526, 7.534, 7.692, 7.701, 7.706 and 7.715 ppm. The observed peaks at 7.512, 7.520, 7.526, 7.534, 7.692, 7.701, 7.706 and 7.715 ppm is due to aromatic ring. This strongly supported the predicted aromatic ring in the FT IR spectrum. The peaks observed at 4.205, 4.220 and 4.234 ppm is due to hydroxyl (OH) vibrations. The peaks at 2853.86 and 2922.89 are due to C – H vibrations of a long chain (figure 2).

3.4 Analysis of ^{13}C NMR spectrum

^{13}C NMR spectrum of our study material showed peaks at 10.953, 14.018, 22,976, 23.781, 28.941, 29.692, 30.392, 38.776, 68.175, 128.804, 130.853, 132.500 and 167.732 ppm. This spectrum indicated the presence of 10 carbon atoms besides the aromatic ring. The peaks at 128.804, 130.853 and 132.500 clearly indicated the presence of an aromatic ring and it also supported the observed peaks in FTIR spectrum. The spectrum also showed a peak at 167.732 which is due to carboxyl group at first carbon atom (figure 3). The next vibration was observed at 68.175 which are due to hydroxyl group (OH) on the second carbon atom. Analysed peaks strongly suggested that the experimental compound may be a phenyl 2 hydroxy decyl ketone (figure 4, 5).

3.5 Antimicrobial activity of *phenyl 2 hydroxy decyl ketone* isolated from *Sansevieria zeylanica* on *Klebsiella pneumoniae*

The inhibition zone diameters produced by *phenyl 2 hydroxy decyl ketone* in two different concentrations clearly indicated their antimicrobial activity. Increased inhibitory zone was developed in the disk impregnated with 50 µl and 100 µl of *phenyl 2 hydroxy decyl ketone* (table 2). The groups treated with *phenyl 2 hydroxy decyl ketone* in two different concentrations of 50 µl and 100 µl were statistically analyzed by calculating the standard error among the IZDs obtained and by Paired Samples T Test. For 5% level of significance, the table value is 3.182. The calculated t value obtained from the experimental group (SI & SII) with negative control groups (SIII & SIV) with positive control group SV were 24.495, 19.596, -1.390 and -1.573 respectively. The calculated t value obtained as negative values indicated that the difference between the experimental groups (SI & SII) with positive control group SV were insignificant (table 3).

No	Phytochemical	Presence
1.	Alkaloid	-
2.	Flavonoid	-
3.	Phenols	+
4.	Tannin	-
5.	Saponin	-

6.	Steroid	-
7.	Terpenoid	-
8.	Carbohydrate	-
9.	Protein	-
10.	Glycoside	-
11.	Reducing sugar	-

\+ Signs denotes the presence
\- Signs denotes the absence

Table: 1 Qualitative analysis of various phytochemicals presents in the indigenous drug *Sansevieria zeylanica.*

Group SI	Group SII	Group SIII	Group SIV	Group SV
0.05 ml of *phenyl 2 hydroxy decyl ketone*	0.1 ml of *phenyl 2 hydroxy decyl ketone*	0.05 ml of 10% DMSO	0.1 ml of 10% DMSO	Standard Antibiotics
11 $\pm$ 0.04082	10 $\pm$ 0.04082	6.0 $\pm$ 0.0000	6.0 $\pm$ 0.0000	17 $\pm$ 4.839

Values shown are mean $\pm$ SE from 4 sets

Table: 2 Antimicrobial activity of *phenyl 2 hydroxy decyl ketone* from *Sansevieria zeylanica* on *Klebsiella pneumonia*

Groups under Comparison	Paired Differences				t	Df	Sig. (2-tailed)
	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
			Lower	Upper			
SI & III	.40825	.20412	4.35039	5.64961	24.495	3	.000
SII &IV	.40825	.20412	3.35039	4.64961	19.596	3	.000
SI & SV	8.63134	4.31567	-19.73439	7.73439	-1.390	3	.259
SII&SV	8.89757	4.44878	-21.15801	7.15801	-1.573	3	.214

Table: 3 Paired Samples Test

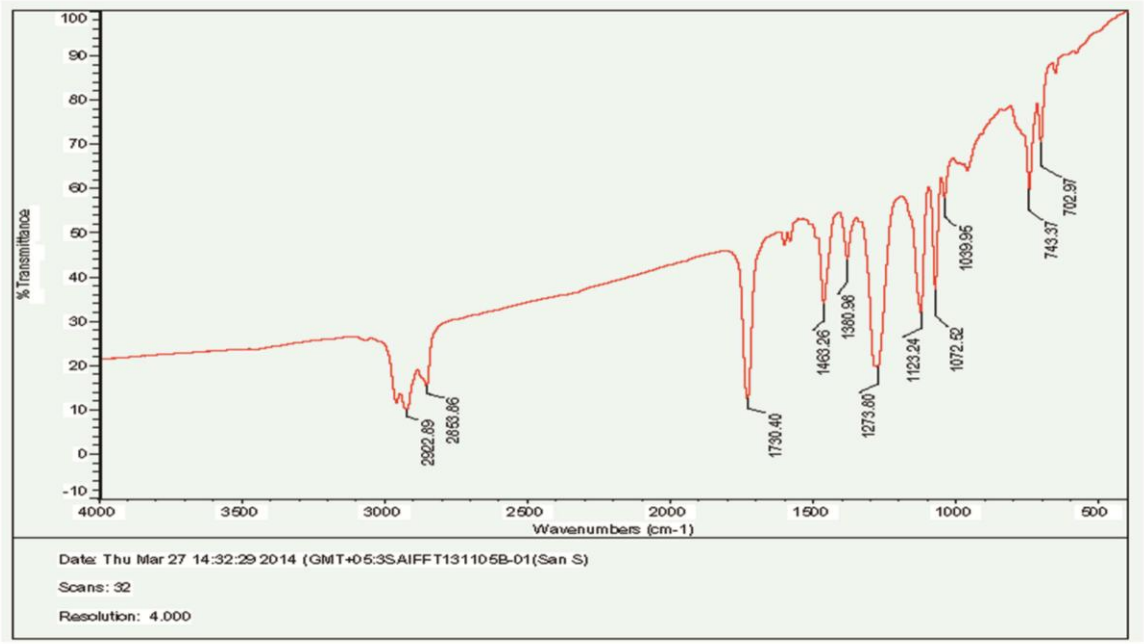

Figure 1. FT IR spectrum analysis of active compound.FT IR spectrum analysis of *phenyl 2 hydroxy decyl ketone* isolated from *Sansevieria zeylanica* showing vibrations of functional groups.

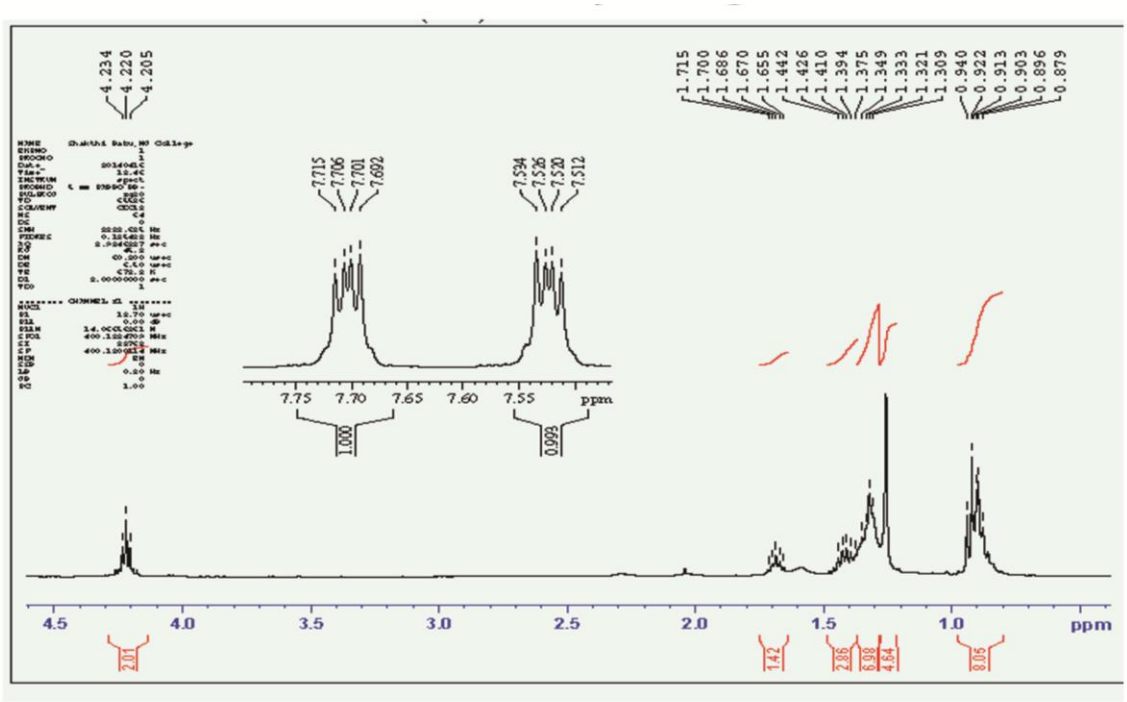

Figure 2. Proton NMR spectrum analysis of active compound. Proton NMR spectrum analysis of *phenyl 2 hydroxy decyl ketone* isolated from *Sansevieria zeylanica* showing the number of hydrogen atoms.

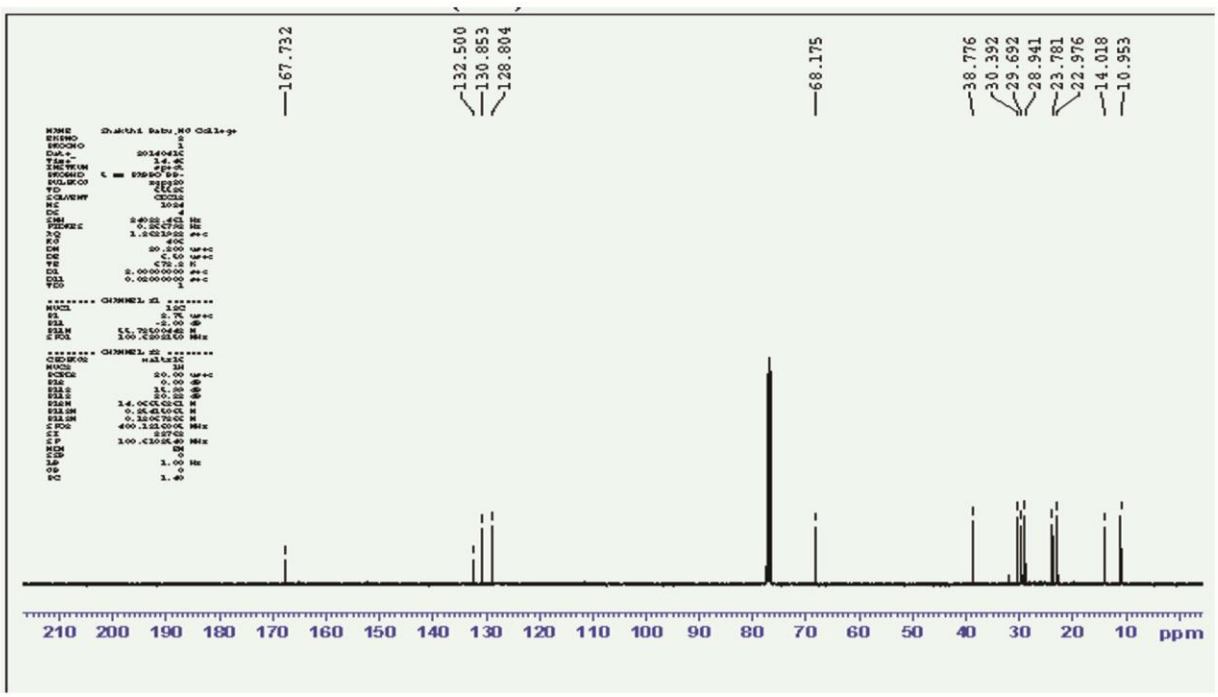

Figure 3.[13]C NMR spectrum analysis of active compound. [13]C NMR spectrum analysis of *phenyl 2 hydroxy decyl ketone* isolated from *Sansevieria zeylanica* showing the number of carbon atoms.

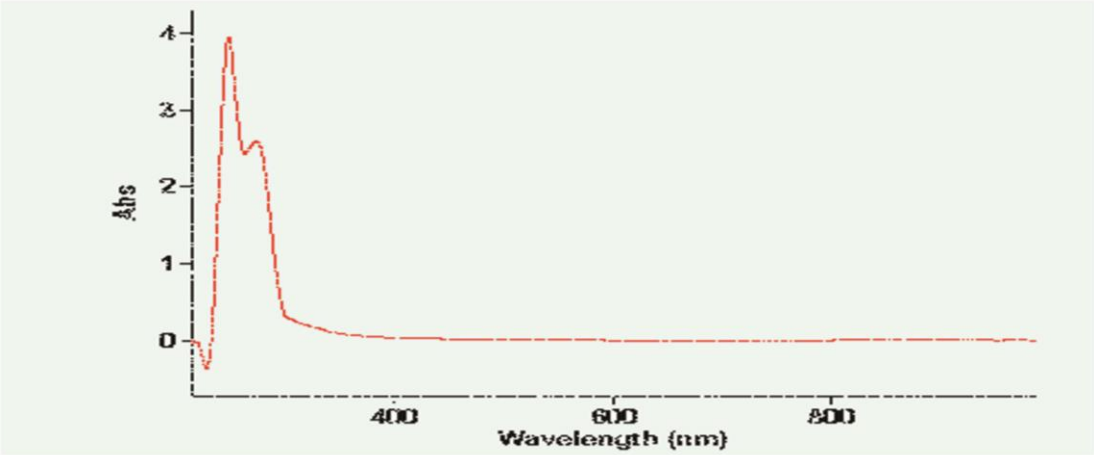

Figure 4. UV spectrum analysis of isolated compound. UV spectrum analysis of *phenyl 2 hydroxy decyl ketone* isolated from *Sansevieria zeylanica shows simple phenol groups.*

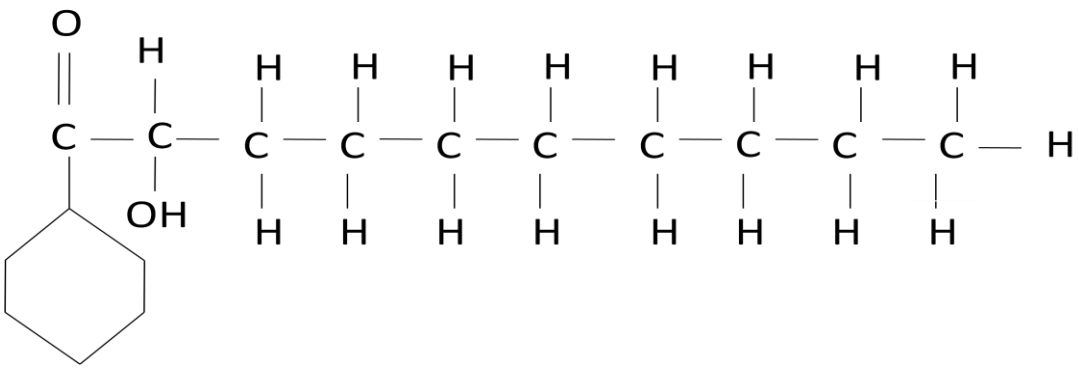

Figure 5. Structure of the isolated compound phenyl 2 hydroxy decyl ketone

4. Discussion

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Numerous studies demonstrated that medicinal plants are sources of nutrient and non-nutrient compounds, many of which display antioxidant and antimicrobial properties which can protect the human body against both cellular oxidation reactions and pathogens. Thus, it is important to characterize the different types of medicinal plants for their antioxidant and antimicrobial potentials, and such plants should be investigated to better understand their properties, safety and efficacy (Nascimento et al., 2000). Ikewuchi et al. (2010) investigated the proximate and phytochemical composition of *Sansevieria liberica* revealed the presence of alkaloids, carotenoids, flavonoids (catechins and flavones), phytates, saponins and tannins and which supported the medicinal use of the plant, and in addition, unveils the possibility of its acting as a potential source of food nutrients and nutraceuticals.

This is the reason why we find research being carried out to isolate the active compounds from the ethyl acetate extract of indigenous drug such as *Sansiviera zeylanica* prescribed in parambharya system of medicines. Phytochemical screening of isolated crystals eluted from ethyl acetate extract of *Sansevieria zeylanica* revealed it is rich in phenols.

The value of UV and visible spectra in identifying unknown constituents is obviously related to the relative complexity of the spectrum and to the general position of the wavelength maxima. If a substance shows a single absorption band between 250 and 260nm, it could be any one of a considerable number of compounds (e.g. a simple phenol, a purine or pyrimidine, an aromatic amino acid and so on). This experiment clearly revealed that the observed UV spectrum showed absorption of 3.948 and 2.593 in the following wave lengths 249 and 274 nm respectively. This strongly supported the predicted crystals isolated from *Sansevieria zeylanica* is having a simple compound phenol.

FT-IR is one of the most widely used methods to identify chemical constituents and elucidate compound structures, and has been used as a requisite method to identify medicines in Pharmacopoeia (Robert, 2014). FT IR spectrum was used to identify functional group of the active components based on peak value in region of infrared radiation.

The region in the IR spectrum above 1200cm-1 shows spectral bands or peaks due to the vibrations of individual bonds or functional groups in the molecule under examination. The region below 1200cm-l shows bands due to the vibrations of the whole molecule and, because of its complexity, is known as the 'fingerprint' region. The fact that many functional groups can be identified by their characteristic vibration frequencies makes the IR spectrum the simplest and often the most reliable method of assigning a compound to its class (Brand and Eglinton, 1965).

FT IR spectrum of our phytochemical study revealed that the major peak at 1730.40 cm-l is due to double bond of carboxyl (C = O) vibrations of ketone, peaks at 2853.86 and 2922.89 are due to C – H vibrations of a long chain and peaks observed in the finger print region of the spectrum (702 and 743.37 cm-1) is due to aromatic ring may be phenol. This results coincides with UV spectral data

Proton NMR spectroscopy essentially provides a means of determining the structure of an organic compound by measuring the magnetic moments of its hydrogen atoms. In most compounds, hydrogen atoms are attached to different groups (as $–CH_2$-, $-CH_3$, -CHO, $-NH_2$, -CHOH-, etc.) and the proton NMR spectrum provides a record of the number of hydrogen atoms in these different situations.

Our study revealed that the ^{1}H NMR spectrum showed absorption peaks in the following wave numbers 4.205, 4.220, 4.234, 7.512, 7.520, 7.526, 7.534, 7.692, 7.701, 7.706 and 7.715 ppm. The observed peaks at 7.512, 7.520, 7.526, 7.534, 7.692, 7.701, 7.706 and 7.715 ppm is due to aromatic ring. This strongly supported the predicted aromatic ring in the FT IR and UV spectrum. The peaks observed at 4.205, 4.220 and 4.234 ppm is due to hydroxyl (OH) vibrations. The peaks at 2853.86 and 2922.89 are due to C – H vibrations of a long chain. Proton NMR data are in agreement with FT IR data.

^{13}C-NMR spectroscopy is essentially complementary to proton NMR and the combination of the two techniques provides a very powerful means of structural elucidation for new compounds. ^{13}C NMR spectrum of our study material showed peaks at 10.953, 14.018, 22,976, 23.781, 28.941, 29.692, 30.392, 38.776, 68.175, 128.804, 130.853, 132.500 and 167.732 ppm. This spectrum indicated the presence of 10 carbon atoms besides the aromatic ring. The peaks at 128.804, 130.853 and 132.500 clearly indicated the presence of an aromatic ring and it also supported the observed peaks in FT IR and UV spectrum. The spectrum also showed a peak at 167.732 which is due to carboxyl group at first carbon atom. The next vibration was observed at 68.175 which are due to hydroxyl group (OH) on the second carbon atom.

Analysed peaks of all structural data obtained from UV, FT IR, ^{1}H NMR and ^{13}C NMR strongly suggested that the experimental compound may be a phenyl 2 hydroxy decyl ketone. This comes to the conclusion that the predicted structure of the isolated compounds may be phenyl 2 hydroxy decyl ketone. Further advanced spectroscopic studies are required for the structural elucidation and identification of other active principles present in the leaves of *Sansiviera zeylanica.*

The results obtained from the biological experiment reveals that inhibition zone diameters produced by phenyl 2 hydroxy decyl ketone in two different concentrations clearly indicated their antimicrobial activity against *Klebsiella pneumoniae*. The disk impregnated with 50 µl and 100 µl of 1mg/ml concentrations showed greater activity irrespective of the concentration of phenyl 2 hydroxy decyl ketone.(Mohana et al., 2008) documented that the warm juice of *Sansevieria zeylanica* leaves is dropped into the ear as a treatment for earache.

Plants produce a vast number of natural compounds (phytochemicals) with diverse antimicrobial potential in order to adapt to these environmental threats. A number of these phytochemicals were extracted & isolated even in ancient times for use in infectious diseases (Williams, 2001; Evans et al., 2002).

In recent times, due to several intricacies of modern antibiotics, there has been significant shift towards alternative treatment and herbal remedies (Patwardhan et al.,2004). Antibiotic screening of plants and natural products used in alternative systems of medicines like Ayurvedic and Unani is a major thrust of R&D, in the Indian pharmaceutical sector today (Afaq et al., 2004; Baijal et al., 2004).

This is the reason why we find research being carried out to screen and investigate traditional herbal preparations and plants prescribed in parambharya system of medicines. The experiments carried out have confirmed the experimental compound may phenyl 2 hydroxy decyl ketone.

The results obtained from the biological work reveals that the eluted compound namely long chain aliphatic ketone with double bond has significant antibacterial activity against *Klebsiella pneumoniae*. This comes to the conclusion that medicinal plants which are traditionally used in Ayurveda or in other herbal medical practices have scientific basics and can be modified to produce specific medicines against *Klebsiella pneumoniae.*

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