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Research Article

Isolation, Characterization and Biological Activities of 1-(5-Hydroxypentyloxy) Propan-2-One from Ethanolic Rhizome Extract of *Kaempferia galanga*

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ABSTRACT

Plants contain several phytocompounds that shows interaction with various proteins involved in diseases. An isolation and characterization of a phytocompound from ethanolic rhizome extract of *K. galanga* was conducted. Characterization of a novel compound was done using UV, proton, ^{13}C, mass and IR spectra's. *K. galanga* contains a novel phytocompound 1-(5-hydroxypentyloxy) propan-2-one identified based on spectra's. Various biological activities like antioxidant, antimicrobial, protease inhibition and anti-proliferation activities were tested for 1-(5-hydroxypentyloxy) propan-2-one. The antioxidant activities with IC_{50} for 1-(5-hydroxypentyloxy) propan-2-one was shown 44.5 µg/ml and IC_{50} for standard (Ascorbic acid) was shown as 24.5µg/ml. The 1-(5-hydroxypentyloxy) propan-2-one were shown good antifungal activities (9 to 12 mm) along with antibacterial activities (10 to 12 mm). The standard antibiotic like Tetracyclin shown zone of inhibition as 10 to 14mm for tested bacteria and for fungi standard as Fluconazole shown zone of inhibition from 13 to 16 mm. The protease inhibition studies for 1-(5-hydroxypentyloxy) propan-2-one of *K. galanga* have shown the good inhibition activity with enzymes like protease K and trypsin. The dose response of 1-(5-hydroxypentyloxy) propan-2-one from K. *galanga* on MCF-7 (Breast Cancer) cell line shown very less activity 130 µgm/ml when compared with the standard tamoxifen 12.5 µgm/ml. Hence the experimentations concluded that a phytocompound, 1-(5-hydroxypentyloxy) propan-2-one from ethanolic rhizome extract of *K. galanga* shown biological activities.

Key-words: Diabetes, Medicinal plants, Chhattisgarh, Disorder, Blood glucose level.

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1. INTRODUCTION

More than 750 medicinal plants from India are described previously by Nair and Mohanan, 1998. The main emphasis is on Ayurvedic, Unani and Sidha systems of herbal medicine [1]. The plants formed the basis for complicated traditional medicine systems like Ayurvedic, Unani and Chinese medicine are evident from formal healing systems. The various diseases are treated from ancient times by using some important drugs still which are derived from these systems. [2].

The medicinal plants used as a source for relief from nearly 130 drugs, all with single chemical entities that are extracted for disease state can be traced back over nearly five millennia in plants, or modified synthetically has been documented in the early civilization in China and India [3]. Nearly 60,000 years ago, ancient civilization from Neanderthals is using medicinal plants that have rich sources of medicines. People from Iraq used plants as antimicrobial agents from ancient times. Plants used medicinally are still widely applied as ethnomedicine all around the world [4]. The pharmacological screening of phytocompounds from ancient times from many herbal industries provides superior medicines for control and cure of various diseases [5].

The nature has been a good source of medicinal agents since times immemorial as virtual compounds in medicine [6, 7]. The bioactive phytocomponents that are isolated and pure active components like alkaloids, anthroquinone, catachol, flavonoids, phenols, saponins, steriods, triterpenoids and tannins give the plant an absolute superiority, safety and competence for biological protection [8,9,10].

A large number of medicinal plants and prescriptions have been mentioned in Chinese and Indian medical literatures aimed to control ageing processes as well in the prevention of diseases for the prolongation of life-span [11, 12]. The history for involvement of plants to medicine is known with reference as sources of novel drug entities such as artemisinin, etoposide and taxol [13]. High through-put robotics have been developed in the search for compounds differentiation from plant extracts associated to chromatographic separation techniques leads to the isolation of biologically active molecules where chemical structures are determining by modern spectroscopic methods [14, 15]. Research and collaboration in phytochemistry is essential for providing good results in control of ageing and disease.

2. MATERIALS AND METHODS

2.1 Collection of plant materials

Fresh plants consisting of rhizomes of *Kaempferia galanga* (Zingiberaceae family) were collected from Visakhapatnam District, Andhra Pradesh and some regions of Kerala during July and August 2011. The plant was authenticated by Dr. P. V. Arjun Rao, Head, Department of Botany, PhytopharmaTechnology Laboratory, Visakhapatnam, India.

2.2 Extraction Process

Nearly 150 g of air dried powder were taken in 900 ml of ethanol and plugged with cotton wool and then kept on orbital shaker for 48 hours with 150 rpm at room temperature. The extracts were filtered with whatmann no 1 filter paper and collect the supernatant. Then solvent evaporated through rotavapour and make the final volume one-fourth of the original volume and stored at the 4^{o}C in air tight containers.

2.3 Isolation and Purification of 1-(5-hydroxypentyloxy) propan-2-one from Rhizomes of *Kaempferia galanga*

Chemicals which are used in this method as Silica gel G (Qualigens, India for TLC) Silica gel G (Qualigens, India for column chromatography). All the chemicals and reagents were used of analytical grade.

The column was packed using n-hexane. The filtrate of ethanol with 5.12gms (w/w) was carried out to Silica gel G-60 (60-120 mesh). Then the column was eluted first with hexane followed by polarity of the system raised by increasing the quantity of ethyl acetate in hexane. Only ethyl acetate used for column eluting for 1-(5-hydroxypentyloxy) propan-2-one, was separated with fractions (F13-F18) with 0.35gms powder 1-(5-hydroxypentyloxy) propan-2-one. 10ml fractions were collected and the fractions having similar compounds were pooled together after monitoring with thin layer chromatography. Visualization of the TLC chromatograms was achieved by using iodine vapors.

The fractions were further subjected to phytochemical screening by using TLC method; those fractions that gave similar spots and Rf with specific TLC methods were again pooled together into a major fraction was 1-(5-hydroxypentyloxy) propan-2-one.

The compounds purity was checked by using n-hexane and ethyl acetate as solvent system on silica gel G TLC plates of 2mm thickness. The iodine chamber was used to visualization of spots. The FT-IR spectra were recorded on perkin-Elmer spectrophotometer. The proton NMR spectra were scanned on a Bruker 400 M Hz. Spectrometer in methanol using TMS as standard and chemical shifts are expressed in δ ppm. The ESI mass spectra were recorded on an Agilent 6100 QQQ spectrometer (positive ion mode & negative ion mode).

2.4 Anti oxidant activity with DPPH

Standard: Ascorbic acid

Chemicals & Reagents:

a. 100μM of DPPH (1,1-diphenyl-2-picrylhydrazyl): About 3.9432 mg of DPPH is added in 3ml of methanol and the content obtained for 100 ml (final concentration 100μM).
b. Stock solutions: About 3 mg/ml of stock solution is equipped in DMSO (or Dimethylsulfoxide).

c. Test solutions: Dilutions from 0 to 1000µg/ml of the test solutions were prepared.

The reaction mixture is prepared in addition of 20 µl of test solutions with 280 µl of DPPH reagent to obtain a final concentration of 300 µl. The solution is kept in incubation chamber in dark for about 50 minutes and the absorbance was taken at 517 nm using the spectrophotometer. An IC_{50} value for anti-oxidant activity was confirmed as elicits that are of half maximal response.

2.5 Antimicrobial Activity

The antimicrobial activity was conducted based on zone method. Microbes from ATCC (American Type Culture Collection), USA have been used in the present study. Several bacteria used in the current research work are *Bacillus subtilis* (ATCC 6051) and *Klebsiella pneumonia (*ATCC 13883) that belongs to gram positive bacteria. *Serratia marcescens* (ATCC 14756), *Pseudomonas aeruginosa* (ATCC 15442), *Enterobacter* aerogenes (ATCC 13048) and *Escherichia coli* (ATCC 25922) belong to gram negative bacteria. The fungi used in the present experimentation are *Aspergillus niger* (ATCC 6275) and *Candida albicans* (ATCC 10231). Antimicrobial activity has been conducted based on zone method.

2.6 Protein Inhibition Activity

Activity for protease inhibitor against proteases (Chymotrypsin, Protease K and Trypsin) was assayed in the present experimentation. In the present method, Trichloroacetic acid (TCA) soluble fractions that formed by action of proteases with protein substrate Hammerstein casein were measured by change in the absorbance at 280 nm. The obtained residual caseinolytic activity for the trypsin in the presence of protease inhibitor at 37˚C was measured with inhibitory activity. Blanks for the substrate, inhibitor and enzyme were included in assay along with test.

Approximately 10 µl of protease inhibitor (plant extract) was mixed with 10 µl of protease (0.5 mg/ml) and was spotted onto a stripe of the X-ray film. 10µl of protease was mixed with 10µl of 0.lM (pH 7.0) phosphate buffer as the control and was spotted on to the X-ray film. The above inhibitor, protein and buffer mixtures were incubated of X-ray film at 37°C for 10 minutes. After 10 minutes, wash the film under tap water gently without touching other objects for the zone of gelatin hydrolysis. The protease activity will be visualized as thick color without gelatin hydrolysis and non-protease activity will be shown as zone of gelatin hydrolysis.

2.7 Antiproliferative Activity

As the ethanolic crude extracts has shown efficient antioxidant, antimicrobial and protease inhibition activities than other tested extracts, a preliminary investigation has been made for finding antiproliferative effects of ethanolic crude rhizome extract of *K. galanga* on MCF-7 (Breast Cancer) cell line.

Human cancer cell line (MCF-7) used in this study were produced from National Centre for Cell Science, Pune. The all cells were grown in Minimal Essential Medium (MEM, GIBCO) and addition with 2mM L-glutamine, 5 percent Fetal Bovine Serum (FBS) (growth medium) and 4.5 g/L glucose at 37˚ C in 5% CO_2 incubator.

The trypsinized cells were seeded in T-25 flask and in every well of 96-well plane-bottomed tissue culture plate at a concentration of $5x10^3$ cells/well in the growth medium were cultured at 37˚C in 5% CO_2 to adhere. After 48 hrs of incubation, the supernatant was redundant and the cells are pretreated with the growth medium. The cells were subsequently mixed with different concentrations of ethanol extracts of *K.galanga* (12.5, 25, 50, 100, 200 and 250 µg/ml) to reach the final volume of 100µl and cultured for 48 hours. The test samples well separately prepared as 1.0 mg/ml concentrations of stock solutions using DMSO. The culture medium and the solvents are used as controls. Each well then added with 5µl of new MTT (i.e 0.5 mg/ml in PBS) left for incubation about 2 hours at 37˚C. The supernatant with the growth medium was then discarded from the wells. The mixture is then replaced with 100µl of DMSO solution to solubilize the formazan product. After 30 min. of incubation, the absorbance or the OD of the culture plate was measured with ELISA reader (Anthos 2020 spectrophotometer model) at a wavelength of 492 nm.

3. RESULTS AND DISCUSSIONS

The phytochemical investigation of the ethanolic rhizome extract of *Kaempferia galanga* was shown good pharmacological activities due to containing following compounds. The TLC result (**Fig.1**) was shown Rf value as 0.8.

Fig. 1. TLC for Column Elute of 1-(5-hydroxypentyloxy) propan-2-one from *K. galanga*

Structure elucidation of ethanolic rhizome elute, 1-(5-hydroxypentyloxy) propan-2-one from *K. galanga* is shown based on the UV, Proton NMR, ^{13}C NMR, Mass and IR spectra's (**Fig. 2 to 6**). **Fig. 7** was shown the structure confirmed based on spectra's.

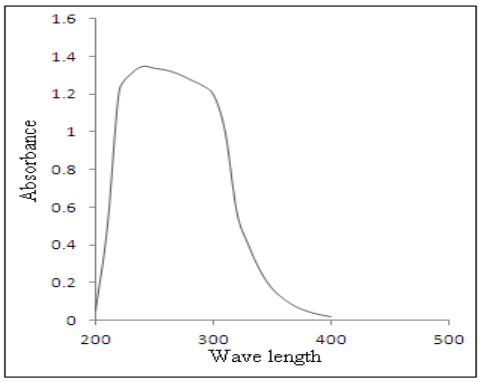


Fig. 2. UV Spectrum for ethanolic rhizome elute, 1-(5-hydroxypentyloxy) propan-2-one from *K. galangal*

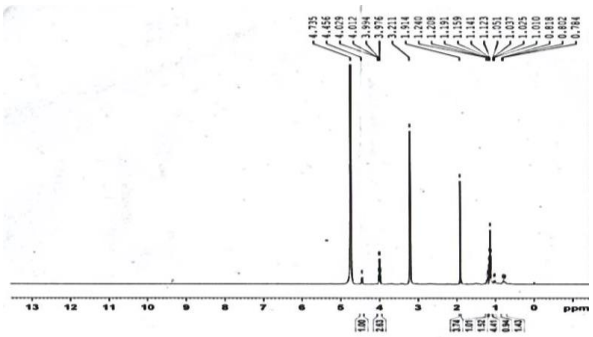


Fig. 3. Proton NMR spectrum for ethanolic rhizome elute, 1-(5-hydroxypentyloxy) propan-2-one from *K. galangal*

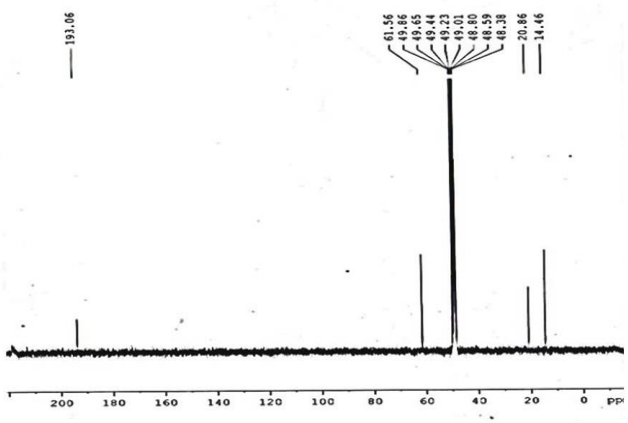


Fig. 4. ^{13}C NMR spectrum for ethanolic rhizome elute, 1-(5-hydroxypentyloxy) propan-2-one from *K. galanga*

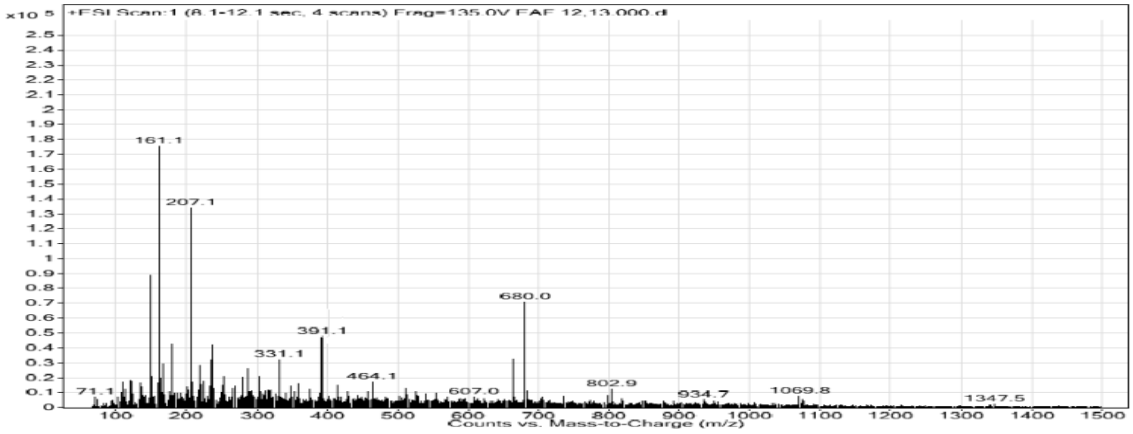


Fig. 5. Mass spectrum for ethanolic rhizome elute, 1-(5-hydroxypentyloxy) propan-2-one from *K. galanga*

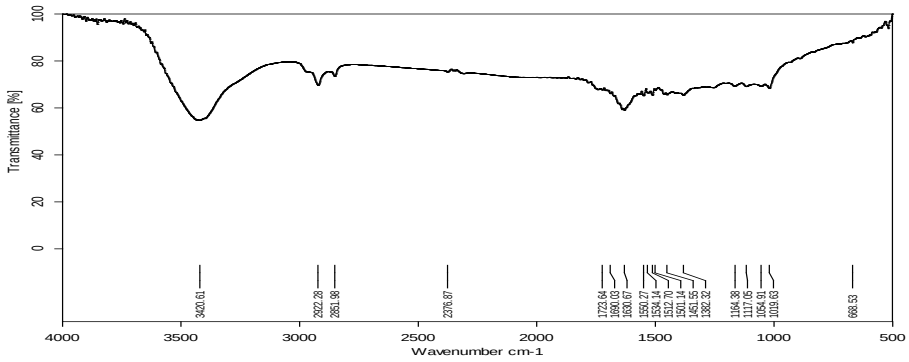


Fig. 6. Infra Red spectrum for ethanolic rhizome elute, 1-(5-hydroxypentyloxy) propan-2-one from *K. galanga*

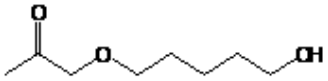
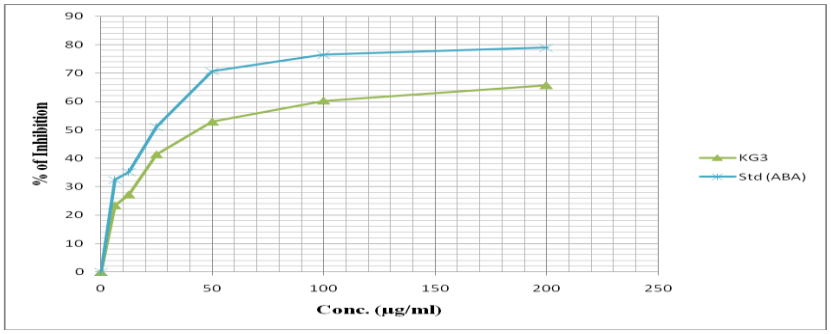


Fig. 7. 1-(5-hydroxypentyloxy) propan-2-one

Table 1 shows the percentage inhibition for antioxidant activities. The IC_{50} for 1-(5-hydroxypentyloxy) propan-2-one was shown as 44.5 µg/ml and IC_{50} for standard was shown as 24.5µg/ml. The antioxidant report was shown that 1-(5-hydroxypentyloxy) propan-2-one has better antioxidant activity (**Fig. 8**).

Table 1. Antioxidant activity of 1-(5-hydroxypentyloxy) propan-2-one and standard

Concentration	1-(5-hydroxypentyloxy) propan-2-one	Standard (ascorbic acid)
0	0	0
31.25	23.44	32.4
62.5	27.23	35.18
125	41.32	51.02
250	52.85	70.63
500	60.23	76.45
1000	65.65	78.94



Note: KG3 stands for 1-(5-hydroxypentyloxy) propan-2-one and std as standard Ascorbic acid

Fig. 8. Antioxidant activity (IC_{50}) for rhizome column elute of 1-(5-hydroxypentyloxy) propan-2-one from *K. galanga*

Medicinal plants produce phytochemicals extensively showing different antimicrobial activities. The antimicrobial activities tested with ethanolic rhizome column elutes of *K. galanga* on different microbes was shown good results (**Table 2).** The 1-(5-hydroxypentyloxy) propan-2-one were shown good antifungal activities (9 to 12 mm) and as well as good antibacterial activities (10 to 12 mm) when compared with bacterial standard like Tetracyclin (Antibiotic) shown zone of inhibitions were (10 to 14mm) and for fungi was Fluconazole shown zone of inhibition were (13 to 16 mm) were low.

Table 2. Antimicrobial activity of rhizome column elute of 1-(5-Hydroxypentyloxy) propan-2-one from *K. galanga*

Microorganisms	Zone of inhibition in mm (including well size of 8mm) at 50 µg/ml	
	1-(5-hydroxypentyloxy) propan-2-one	Antibiotic
Bacteria		
B. subtilis	11	12
K. pneumoniae	11	10
S. marcescens	12	14
P. aeruginosa	10	11
E. aerogenes	10	10
E. coli	11	10
Fungi		
A. niger	9	13
C. albicans	12	16

Note: Antibiotic used for bacteria is Tetracyclin and fungi is Fluconazole

The protease inhibition studies for 1-(5-hydroxypentyloxy) propan-2-one of *K. galanga* have shown the good inhibition activity with enzymes like protease K and trypsin when compared with chymotrypsin (**Table 3 and Fig. 9**). The compound 1-(5-hydroxypentyloxy) propan-2-one of *K. galanga* was not shown inhibition with chymotrypsin based in X-ray photographic film and *in vitro* assay methods. The standard tetracycline had shown the complete inhibition with proteolytic enzymes and control was not shown the protease inhibition (formed zone) with proteolytic enzymes on X-ray photographic film.

Table 3. IC_{50} values for protease inhibitors (in µM) from *K. galanga*

Compound	Protease K	Trypsin	Chymotrypsin
1-(5-hydroxypentyloxy) propan-2-one	32.33±0.88	24±0.58	42±0.58
Tetracycline(Standard)	22.2±0.66	20.1±0.58	24.6±0.58
Control (Phosphate Buffer)	0	0	0

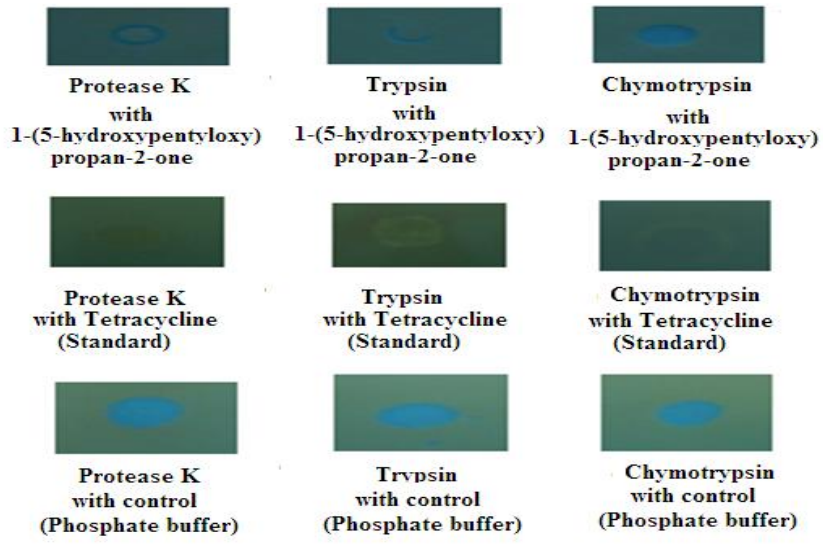


Fig. 9. Protease inhibition assay using X-ray film method for 1-(5-hydroxypentyloxy) propan-2-one of *K. galanga*

The dose response of 1-(5-hydroxypentyloxy) propan-2-one from *K. galanga* on MCF-7 (Breast Cancer) cell line shown very less activity 130 µgm/ml when compared with the standard Tamoxifen 12.5 µgm/ml (**Table 4 and Fig. 10**).

Table 4. Dose response of 1-(5-hydroxypentyloxy) propan-2-one *K. galanga* on MCF-7 (Breast Cancer) cell line

Conc in (Micro gm/ml)	% of Cell survival for Tamoxifen	% of Cell inhibition for Tamoxifen	% of cell survival for 1-(5-hydroxypentyloxy) propan-2-one	% of cell inhibition for 1-(5-hydroxypentyloxy) propan-2-one
0	100	0	100	0
6.25	82.3	17.7	98.9	1.1
12.5	45.5	54.5	93.9	6.1
25	30.9	69.1	83.7	16.3
50	16	84	67.6	32.4
100	4.9	95.1	56.5	43.5
200	0.4	99.6	35.8	64.2

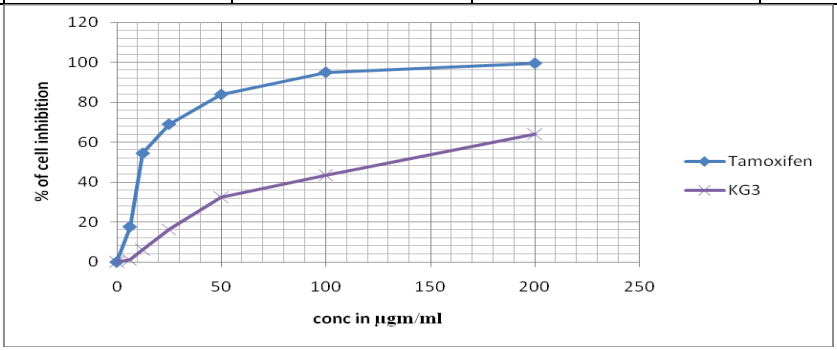


Fig. 10. Antiproliferative activity of 1-(5-hydroxypentyloxy) propan-2-one from *K. galanga*

The plants of special interest are used in the development of pharmaceuticals all over the world [16, 17]. The ethnopharmacological studies will results in the beginning of new solitary molecule drugs that can have a greater role to play if the crude extracts are established for clinical use in the world [18].

The extensive benefits in developing countries towards modern medicine are potential when the local available medicinal plants are focused to scientific methods of confirmation of traditional use and quality control towards modern biotechnology approaches [19]. Hence the approach is provided achievement in several parts of the world but is not always esteemed by international agencies and national governments [20, 21]. As a result, plants used in traditional medicine play a vital role in the maintenance of healthiness in some parts of the world in the introduction of new treatments [22, 23]. *K. galanga* leaves possessed anti-inflammatory and antinociceptive activities and thus supports the Malay's traditional uses of the plant for treatments of mouth ulcer, sore throat, headache, etc [24].

4. CONCLUSION

The ethanolic extract of *K. galanga* contains a phytocompound 1-(5-hydroxypentyloxy) propan-2-one that shows antioxidant, antimicrobial, protease inhibition and anti-proliferation activities,

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