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Isolation, Characterization, and Anti-cancer Activity of Kaempferol-6-methoxy-7-*O*-glucoside from *Lantana camara* Flowers

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ABSTRACT

Traditionally, many indigenous plants are identified for their impending supply of medicinal compounds which are used in traditional medicine. Herein, the *In-vitro* anti-cancer property of Kaempferol-6-methoxy-7-*O*-glucoside was determined isolated from *Lantana camara* flowers. This compound was characterised through ^{13}C, $^{1}HNMR$, FT-IR and UV-spectroscopy. *Lantana camara* flowers have been found the best anti-cancer activity due to absolute best flavonoids content. This medicinal plant has potential flavonoids composition which should supply scientific evidences for the usage in the remedy of most cancers.

Keywords: *Lantana camara* flowers, Flavonoids, Anti-cancer activity.

INTRODUCTION

The universe has constituted many eco-systems in which researchers are exploring the valuable medicinal plants indigenously. The green plant is essential to all extraordinary life. The oxygen we breathe, the nutritional vitamins we consume, the fuels we burn and many of the most essential substances we use which are produced from the resource of plants. Association between plant and man is an age-old system starting from human civilization. Since time immemorial, nature has been giving a wealthy contribution of medicinal constituents. Using the well-known knowledge there are isolation of phytoconstituents carried out from herbal sources[1-3]. The magnitude of vegetation as one of the herbal sources of drugs cannot be overemphasized as about 25% of the drugs prescribed world come from plants[4]. Ethno botany has developed as an essential research self-discipline as plant life continues to be an quintessential part of human existence[5,6]. India is one of the resourceful countries in the word which has valuable plant kingdom. Indigenously, many plants are very useful for finding medicinal constituents which are applied in various fields including pharmacological, pharmaceutical, biological etc[7-10]. People have adopted the historic illnesses healing structures like Ayurveda, Siddha and Unani to maintain away from unfavorable factor outcomes

of artificial drugs[11-13]. The herbal formulations had been used with the beneficial resource of the natural medicinal practitioners except perception the plant compounds and these practices have been continued technology to generations barring documentation. Therefore, to be aware of the plausible of a plant crude drug phytochemical evaluation is very essential.

Extraction and fractionation of Kaempferol-6-methoxy-7-*O*-glucoside from *Lantana camara* flowers

Extraction and fractionation

Fresh flora of *Lantana camara* have been gathered at Thiruppanandal village, Thanjavur District, Tamil Nadu in the path of December had been extracted with ninety five percentage methanol under reflux. The isolation of the methanol extract used to be positioned in column chromatography alongside with silica gel (60-120 mesh) as a stationary phase. The charged column was once as soon as then eluted with one of a variety mobile phases with gradual prolong in polarity. The fractions have been accumulated and the solvent recovered through the use of convenient distillation. All the concentrated fractions have been subjected to Thin Layer Chromatography (TLC) for the identification of the preferred bands.

Thin Layer Chromatography used to be carried out on the 20 X 20 cm plates precoated with silica gel (Sigma Aldrich Co., India). TLC comparison of ether and ethyl acetate extracts had been carried out the utilization of three distinct developing solvent systems (BuOH-AcOH-H_2O, 4:1:5 (in volume); EtOAc-HCOOH-H_2O, 10:2:3 (in volume); 15% AcOH. Based on the Rf-value, extent of fractions had been obtained and the one with extremely good selection was once as soon as visualized under ultraviolet (UV) light, indicating that it used to be a pure compound, used to be selected. The ether fraction used to be concentrated *in vacuo* and left in an ice-chest for seven days. The residue from the ether fraction of the hydrosylate was once taken up in acetone and left underneath chilled conditions for a few days when yellow needles (Melting point: 275-277°C) had been obtained. It viewed yellow colourunder ultraviolet (UV) light with ammonia. It responded to Horhammer-Hansel, Wilson's boric acid, Gibb's and Shinoda tests and not a response to Molisch's test.

Ethyl acetate fraction: Kaempferol-6-methoxy-7-*O*-glucoside

The ethyl acetate fraction used to be once concentrated *in vacuo* and left in an ice-chest for a few days. A yellow solid that separated used to be once filtered and studied. It got here out as pale yellow crystals (m.p. 242-244°C) on recrystallization from methanol. It gave greenish brown colour with alc. Fe^{3+}, an immoderate yellow coloration with NaOH, purple colour with Mg-HCl and yellow precipitate with aqueous Lead acetate. It considered dull yellow colourunder ultraviolet light with ammonia and it responsible to Wilson's boric acid, Horhammer-Hansel, Gibb's and Molisch's tests. Pale yellow needles, m.p. 242-244°C, λ^{MeOH}_{max} 260, 335nm; IR (KBr, ν_{max}, cm^{-1}): 3600, 3228, 2919, 1679, 1621, 1599, 1541, 1496, 1473, 1409; ^{1}H-NMR (400 MHz, DMSO-*d6*): δ 16.800 (br s, 1H, 5-OH), 12.22 (br s, 1H, 3-OH), 9.30 (br s, 1H, phenyl C_5-OH), 7.69 (d, J=6.6 C_2 & C_6-H), 7.53 (d, J=8.4, C_3 & C_5-H), 5.45 (s, 1H, Chromen C_8-H), 4.99 (s, 1H, pyranose-C_3-OH), 4.97 (s, 1H, pyranose C_4-OH), 4.64 (s, 1H, pyranose C_5-OH), 3.75 to 3.17 (unresolved pyranose and ethylene proton), 3.44 (s, Chromone-$_3$H, OCH_3); ^{13}C-NMR (100 MHz, DMSO-*d6*): δ 176.17, 176.09, 159.25, 151.54, 148.10, 147.75, 147.48, 147.42, 145.33, 145.00, 135.56, 129.62, 129.54, 122.00, 121.71, 119.91, 115.51, 115.41, 115.35, 105.08, 100.92, 93.61, 93.56, 77.24, 76.21, 75.78, 73.16, 69.66, 60.63, 56.00; GC-MS: m/z [M+1] 478.

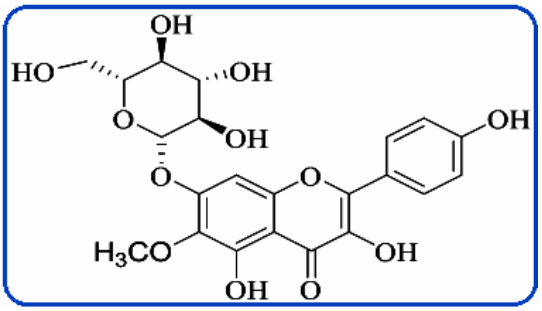

Fig. 1. Structure of Kaempferol-6-methoxy-7-O-glucoside

RESULT AND DISCUSSION

The clean flowers of *Lantana camara* have been discovered Kaempferol-6-methoxy-7-*O*-glucoside (Fig.1). Pale yellow crystal; m.p. 242-244°C.

UV-Spectroscopy

The Ultraviolet (UV) spectrum (Fig. 2) of the glycoside was once exhibited fundamental peaks at 230, 260, 335nm to expose flavonol skeleton.

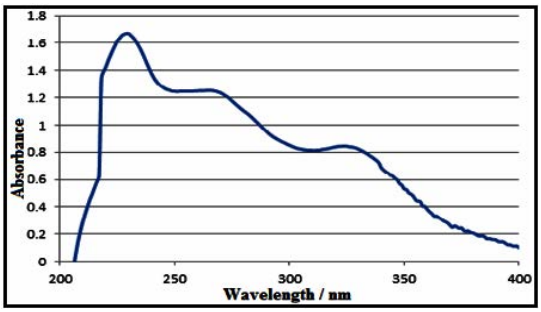

Fig. 2. UV-Spectrum of Kaempferol-6-methoxy-7-O-glucoside

The majority of flavonoid compounds have some fundamental absorption bands which are shown in the ranges of 300-400 and 240-285nm. The predominant absorption band seems in the range of 230-350nm. The bathochromic shift in flavonols can be ascribed to the smallest HOMO-LUMO gap, which has some final result of the existence of the O_3--H_3--O_4 hydrogen bond. The strongest bands have been found as 260 and 335nm in UV spectrum of glycoside compound respectively which have exposed the presence of different hydroxy and methoxy groups in the glycoside structure.

^{1}H-NMR spectroscopy

The ^{1}H-NMR spectrum (Fig. 3) confirmed flavonoid skeleton C5 hydroxy protons show up broad singlet at δ 12.2ppm. Chromone-2-substituted phenyl ring 5-hydroxy group proton show up broad singlet at δ 9.30ppm and doublet for two ortho coupled proton for δ 7.69ppm in addition two meta coupled doublet at δ 7.522ppm. The chromone building C8-H proton singlet peak show up at δ 5.45 ppm. The pyranose C_2-linkage proton show up sign δ 4.99 ppm for doublet, pyranose existed three C_3, C_4 and C_5 hydroxy proton broad singlet in order of δ 4.99, 4.97 and 4.64ppm, moreover glucose moiety proton indicates at unresolved peak existing at the vary of δ 3.75 - 3.17ppm. The chromone C_6 building of aliphatic of -OCH_3 as a singlet at δ 3.44ppm.

^{13}C-NMR spectroscopy

^{13}C-NMR spectrum (Fig. 4) chemical shift of the carbon signals at δ 176.17ppm confirmed the presence of C=O group and additionally confirmed (C-2′, C-6′) δ 105.08, (C-3′, C-5′) δ 100.92ppm, which relatively corresponded with these of hydrogen bearing carbons of p-cresol (δ 115.35, 115.41 and 115.51ppm) and moreover verified the presence phenyl carbon (C-2) at δ 159.25ppm and oxygen bonded ethylene carbon (C-3) at δ 135.56ppm. The glycosylation at the third position (C-3), C-2 and C-4 carbons assimilate at δ 148.10ppm and δ 73.16ppm respectively. The C-6 and C-8 carbons show up regions at δ 122.00 and δ 93.68ppm respectively. The C-1″ of glucose looks at δ 119.91ppm and the leisure of the methoxy carbon exhibit up in the range δ 60.63 and δ 56ppm verified the structure of Kaempferol-6-methoxy-7-*O*-glucoside[14] in a similar way helps mass spectrum of Kaempferol-6-methoxy-7-*O*-glucoside as molecular formula $C_{22}H_{22}O_{11}$ m/z (%): 479 [M+1] (48%).

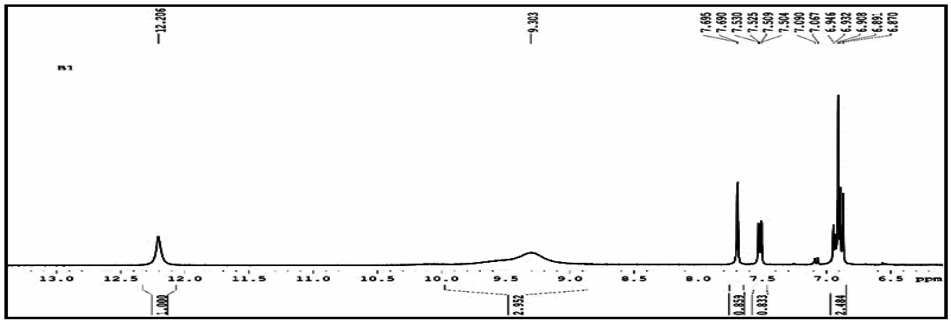

Fig. 3. ^{1}H-NMR Expand spectrum of Kaempferol-6-methoxy-7-*O*-glucoside

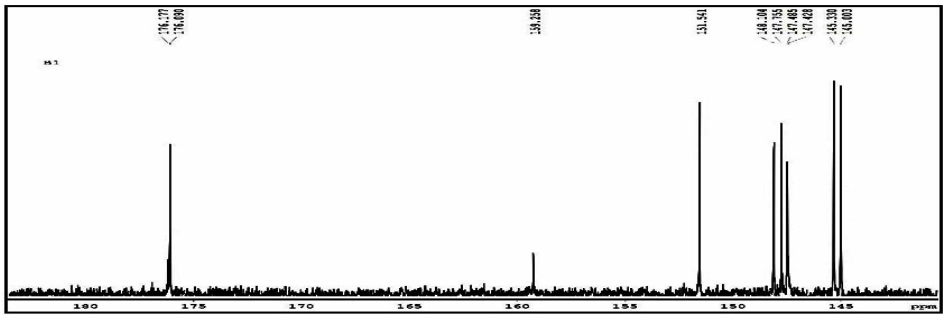

Fig. 4. ^{13}C-NMR Expand spectrum of Kaempferol-6-methoxy-7-*O*-glucoside

Anti-cancer activity

The flavonoid glycosides of each and every concentration used to be carried out in quadruplicate and cumulative variant have been maintained lots much less than 20% between the facts points. Three set of cell strains had been examined in a 96-well plate as described in the underneath 96-well format. The isolated Kaempferol-6-methoxy-7-*O*-glucoside was once exhibited common inhibition in HeLa cell lines with GI_{50} of 33.6 μg, TGI of >100 and LC_{50} of >100 respectively. It has been stated that this drug can also choose to moreover be used for the treatment of colon, breast cancer, after subsequent experimental validations, due to its strong interaction with all the protein PDB structures of the gene. A thorough comparative study used to be as soon as carried out, to look at the evidence that existed after the inhibition of this protein product. All of the proof suggests that inhibition of the expression of the gene, especially in tumour cells, would decrease the improvement and growth of the tumour which is illustrated in Fig. 6. The Percentage growth of HeLa in the direction of the flavonoid glycoside consequences and raw facts has been illustrated in Table 1. Fig. 5, illustrates the pictorial illustration of a more advantageous efficacy of anti-cancer recreation of Kaempferol-6-methoxy-7-*O*-glucoside.

Table 1: Percentage growth of HeLa against the flavonoid glycoside

Name of the compound	Percentage growth(μg)					Growth Inhibition in μg		
Kaempferol-6-methoxy -7-*O*-glucoside	100 μg	10 μg	1 μg	0.1 μg	0.01 μg	GI_{50}	TGI	LC_{50}
	20	83	102	110	103	33.6	>100	>100

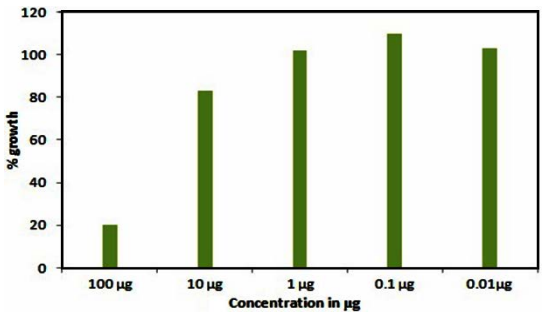

Fig. 5. Diagram of percentage growth of HeLa against the flavonoid glycoside

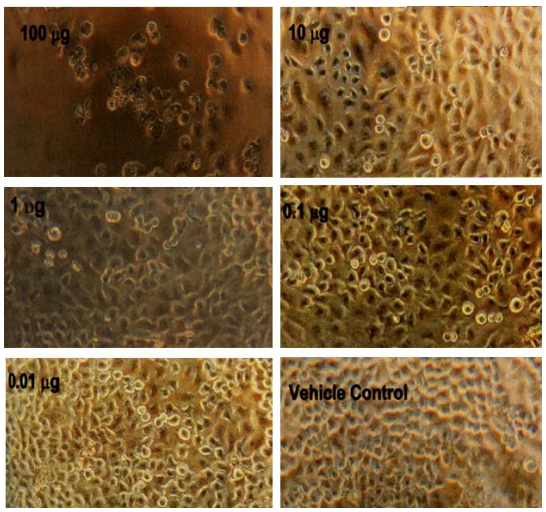

Fig. 6. HeLa cells treated with the flavonoid glycoside G3 for forty eight hours Kaempferol-6-methoxy7-*O*-glucoside (G3) treated HeLa cells

CONCLUSION

In recent years many substances from plants extracts are utilizing for various therapeutic uses. The flavonoid compounds have potential cytotoxicity with suitable mechanisms of action; like inhibition of tumour cell growth. The flavonoid compound Kaempferol-6-methoxy-7-*O*-glucoside was isolated from *Lantana camara* flower has proved its efficacy against the growth of cancer cells. Moreover, the anticancer effect of the compound wasshown that extracted compounds from plant whichis performed as a potential candidate to destroy tumour cell without toxic effect to the normal cell.

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Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

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