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Isolation and Characterization of Some Pharmaceutical Active Flavonoids from Cichorrium Intybus Plant (Kasini)

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Abstract

Due to the biological activity shown by many natural products, these studies describe the isolation of some pharmaceutical active compounds (flavonoids) that may be used by humans to health benefits. The study contains the isolation and characterization of active pharmaceutical compounds from Cichorrium Intybus (Kasini). The isolated compounds are characterized on the basis of UV-Vis Spectrophotometer, IR, NMR (^{1}H & ^{13}C) and mass spectral data.

Introduction

Plants have been used as traditional remedies for the treatment of different ailments. The herbal health care traditions have evolved over centuries as relevant social traditions with evidenced safe, efficacious, preventive, promotive and curative health practises. World health organization (WHO) has recognized the importance of medicinal plants in health care system and shown great interest in documenting the medicinal plants used by tribal people[1]. Plants are an excellent source for various drugs because most of the natural drugs have lesser side effects. All parts of the plant have medicinal properties. Precedence exists in literatures, the various biological activity of *Cichorium intybus* may help humans with weight loss, constipation, improving bowel function and general health.[2-4] Recently, the reported literature (Mohit *et al.*, 2012), it has been observed that alcoholic extract of these plants has appreciable immunomodulator activity[5-12]. Hence, alcoholic extract of these plants are column chromatographed in an attempt to isolate the active compounds. So far to our knowledge there was no method has been reported for isolation and characterization of these isolated compounds.

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Experimental

Isolation of compounds from cichorrium intybus (kasini) :

The method of preparation of alcoholic extract was reported by mohit *et al*. The alcoholic extract was column chromatographed. The column was then eluted successively with hexane, hexane: chloroform (1:1), chloroform, chloroform: methanol (1:1), and methanol. Every fraction of 250 ml was collected and concentrated to a small volume and four major fractions (I 5.59 g, II 6.41 g, III 10.93 g, and IV 17.74 g) were separated by monitoring with TLC (2 x 5 cm^2 in size with chloroform: methanol, 9:1 as developing solvent) in order to combine the fractions with the same compounds. Each fraction was then examined by TLC (4 x 5 cm^2 in size with chloroform: methanol, 9:1 as developing solvent); using iodine vapours as detecting agent.

The fraction I and II could not be processed due to the complex mixture of so many compounds. A portion of fraction III (1.585 g) was further separated by preparative TLC (20 x 20 cm^2 in size, coated with methanol suspension of silica gel 60 with layer thickness of 2 mm and chloroform: methanol, 9:1 as developing solvent). Two fractions (A 0.193 g and B 0.169 g) were afforded. Fraction A was further purified by preparative TLC using the same developing solvent to give crude compound 1 (0.0905 g), which was recrystallized from chloroform-methanol mixed solvents to obtain pure compound MKD-**1** (0.085g) as light yellow powder.

Fraction IV (14.2 g) was further separated using a column (3 cm in diameter and 30 cm in length) packed with hexane slurry of silica gel 60. The column was eluted successively with hexane, hexane: chloroform (1:1), chloroform, chloroform: methanol (1:1), and methanol. Every fraction of 100 ml was collected and concentrated to a small volume. A portion of methanol fraction (1.0099 g) was further separated by preparative TLC (20 x 20 cm^2 in size, coated with methanol suspension of silica gel 60 G with layer thickness of 2 mm and chloroform: methanol, 8:2 as developing solvent) to afford three fractions (C 0.210 g, D 0.327 g, and E 0.415 g).

Fraction C (0.210 g) was further purified by preparative TLC using the same developing solvent to give crude compound 2 (0.096 g), recrystallized from methanol to obtain pure compound MKD-2 (0.085 g) as light yellow powder.

Fraction D (0.327 g) was further purified by preparative TLC (the plate preparation was as usual) using the same developing solvent to give crude compound 3 (0.090 g), which was recrystallized from methanol to obtain pure compound MKD-3 (0.076 g) as bright yellow powder. The structures of isolated flavonoids (MKD-1, MKD-2 & MKD-3) are shown in fig. 01.

Characterization of the isolated compounds:

UV-Vis spectrophotometer analysis: it was performed using shimadzu 1610 system using chloroform as vehicle solvent.

Infra red spectra: infrared spectra were recorded on a perkin elmer spectrum FT-IR spectrophotometer using KBr pellets in the

range of 4000-450cm^{-1}

NMR spectra: 1H and 13C NMR spectra were recorded on 400 MHz bruker spectrometer in DMSO-d6 using tetramethyl-silane as internal standard at 23^{o}C.

Mass spectra: the electrospray ionization mass spectra (ESI MS) were recorded using water Q-TOF mass spectrometer. The sample was dissolved in methanol and introduced into the ESI source through a syringe pump at the rate of 10µl per minute.

Result and Discussion

The three important fractions (MKD1, MKD2 & MKD3) were isolated from kasmini plant and characterized by using UV-Vis spctophotometer, IR, Mass Spectrometer, 1H & 13C NMR. The characterization of the structures of the isolated compounds was established on the basis of NMR spectra by comparison of their NMR spectral data with literature values. The structure of isolated compounds has been shown in fig. 01. The structure elucidation and characterization data of three different isolated compounds are as follows:

1. Compound MKD-1

The characteristic light yellow and the UV spectrum of compound, MKD-1 suggested that compound, MKD-1 is a flavonoid derivative compound.

The IR spectrum of compound, MKD-1 has important absorptions were attributable to hydroxyl (3607-3084 cm^{-1}) and carbonyl functions (1659 cm^{-1}). Other peaks were assigned as shown in Table 1.

The ^{1}H-NMR and ^{13}C-NMR spectra of compound,MKD-1 showed the necessary diagnostic peaks to be identified as a flavonoid derivative with hydroxyl group on C-3, C-5 and C-7, appearing as sharp singlets at δ 9.59, 12.43 and 10.74 ppm, respectively. The H-6 and H-8 occurred as sharp doublet at δ 6.21 and 6.43 ppm, respectively (JH8/H6= 1.53 Hz). The B ring signals were easily assigned by consideration of symmetry. The H-2' and H-6' resonances occurred as sharp doublet at δ 8.20 ppm. The H-3' and H-5' resonances appeared as doublet of doublets at δ 7.62 ppm and H-4' occurred as muiltplet at δ 7.53 ppm. The 13C experiments of compound 1 gave thirteen peaks. The most downfield shifted peak was 178.1 ppm which was assigned as ketone group (C-4). Compound 1 exhibited the 1H-NMR and 13C-NMR chemical shifts identical to 3,5,7-trihydroxyflavone as shown in Table 2 and 3 with comparison of its spectral data with previous reports.

Further studies of the structure of compound 1 were done by analysis of the high resolution EI-MS. The Mass spectra showed the [M+H^{+}] and [M-H^{-}] peaks at m/z = 271 and 269, respectively.

2. *Compound MKD-2*

The characteristic light yellow and the UV spectrum of compound, MKD-2 suggested that compound 2 is a flavonoid derivative compound similar to compound, MKD- 1. The IR spectrum of compound, MKD- 2 has been showed important absorptions were attributable

Table 1. UV, IR absorption band assignments, NMR (1H & 13C) & of compounds *ie.*, MKD- 1 MKD-2 & Kaempferide (Standard)

S. No.	INSTRU-MENTS	MKD1		MKD2		Kaem-pferide
	UV λmax	values are 267 and 370 nm		values are 272, 315 and 378		370nm
	IR (cm^{-1})	3607-3084 (s) O-H Strec. 1659 (m) C=O Strec. 1600, 1550 (m) C=C Strec. 1659 (m) C-H Bend.		2950, 2875 (m) C-H Strec. 1640 (s) C=O Strec. 1610, 1425 (s) C=C Strec. 1125 (m) C-H Strec.		----
	^{1}H-NMR	**Proton position**	**Chemical shift in ppm(δ_H)**	**Proton position**	**Chemical shift in ppm(δ_H)**	**Chemical shift in ppm(δ_H) (kaemp-feride)**
		3-OH 5-OH 6 7-OH 8 2’ 3’ 4’ 5’ 6’	9.59(s) 12.43(s) 6.21(d) 10.74(s) 6.43(d) 8.20(dd) 7.62(dd) 7.53(m) 7.62(dd) 8.20(dd)	3-OH 5-OH 6 7-OH 8 2’ 3’ 4’-OCH_3 5’ 6’	9.41(s) 12.33(s) 6.25(d) 10.81(s) 6.41(d) 8.07(d) 7.05(d) 3.79(s) 7.05(d) 8.07(d)	9.47(s) 12.43(s) 6.29(d) 10.83(s) 6.45(d) 8.12(d) 7.09(d) 3.81(s) 7.09(d) 8.12(d)
	^{13}C-NMR	**Carbon position**	**Chemical shift in ppm(δ_c)**	**Proton position**	**Chemical shift in ppm(δ_H)**	**Chemical shift in ppm(δ_H) (kaemp-feride)**
		2 3 4	159.8 136.4 178.1	2 3 4	146.0 135.9 176.0	146.3 136.1 176.1

		5	169.1	5	160.5	160.8
		6	98.1	6	98.1	98.3
		8	97.8	7	163.7	164.1
		10	159.7	8	93.3	93.6
		1’	130.2	9	156.1	156.3
		2’	126.1	10	103.4	103.7
		3’	128.5	1’	123.0	123.3
		4’	127.9	2’	129.2	123.3
		5’	128.5	3’	114.0	114.1
		6’	126.1	4’	160.3	160.6
				5’	114.0	114.1
				6’	129.2	129.4
				4’-OCH_3	55.1	55.4
	Mass spectro-meter (m/z)	**271 $[M+1]^+$ 269 $[M-1]^-$**		---		---

Table 2. UV-Vis, IR absorption band assignments of compound *ie.*, MKD- 3

S.No	**INSTRUMENTS**	**MKD3**
	UV (λmax)	values are 270 and 390 nm
	IR (cm^{-1})	3650-3200 (s) O-H Strec. 2970 (m) C-H Strec. 1639, 1550 (m) C=O Strec. 1635, 1500 (s) C=C Bend. 1200 (m) C-O Bend.

to hydroxyl (3650-3300 cm^{-1}) and carbonyl (1640 cm^{-1}) groups. Peaks at 2950 and 2875 cm^{-1} were assigned as sp3 C–H, 1640 cm^{-1} as C=O, 1610 and 1425 cm^{-1} as conjugated C=C aromatic ring, and 1125 cm^{-1} as C–O as shown in Table 4.

The ^{1}H-NMR and ^{13}C-NMR spectra

Table 3. ^{1}H-NMR chemical shifts of compound MKD-3, and kaempferide-3-O-β- glucoside and kaempferol-3-O-β-D-glucoside

Carbon Position	Chemical Shifts in ppm (δ_H)		
	Compound 3	Kaempferide-3-*O*-*β*-D-glucoside	Kaempferide-3-*O*-*β*-D-glucoside
5-OH	12.41 (*s*)	12.45 (*s*)	12.53 (*s*)
6	5.95 (*s*)	5.98 (*s*)	6.11 (*s*)
7-OH	10.69 (*s*)	10.79 (*s*)	10.81 (*s*)
8	6.07 (*s*)	6.18 (*s*)	6.23 (*s*)
2^1	8.03	8.09	8.14
	(*d*, *J*=8.72 Hz)	(*d*, *J*=8.96 Hz)	(*d*, *J*=8.80 Hz)
3^1	7.01	7.05	7.09
	(*d*, *J*=8.72 Hz)	(*d*, *J*=8.96 Hz)	(*d*, *J*=8.80 Hz)
4^1- OCH_3	3.80 (*s*)	3.83 (*s*)	-
5^1	7.01	7.05	7.09
	(*d*, *J*=8.72 Hz)	(*d*, *J*=8.96 Hz)	(*d*, *J*=8.80 Hz)
6^1	8.03	8.09	8.14
	(*d*, *J*=8.72 Hz)	(*d*, *J*=8.96 Hz)	(*d*, *J*=8.80 Hz)
1^2-glu	5.37	5.40	5.42
	(*d*, *J*=7.90 Hz)	(*d*, *J*=7.90 Hz)	(*d*, *J*=7.90 Hz)
2^2	3.21 (*m*)	3.30 (*m*)	3.33 (*m*)
3^2	3.30 (*m*)	3.35 (*m*)	3.41 (*m*)
4^2	3.13 (*m*)	3.18 (*m*)	3.25 (*m*)
5^2	3.42 (*m*)	3.45 (*m*)	3.53 (*m*)
6^2	3.42 (*m*)	3.45 (*m*)	3.54 (*m*)

Table 4. ^{13}C-NMR chemical shifts of compound, MKD-3, kaempferide-3-O-β-Dglucoside and kaempferol-3-O-β-D-glucoside

Carbon Position	Chemical Shifts in ppm (δ_H)		
	Compound 3	Kaempferide-3-*O*-*β*-D-glucoside	Kaempferide-3-*O*-*β*-D-glucoside
2	155.7	155.92	155.87
3	133.0	133.06	132.76
4	176.8	176.94	176.91
5	160.6	160.66	159.42
6	98.2	98.37	98.33
7	163.6	163.66	163.60
8	93.3	93.35	93.30
9	155.3	155.35	155.75
10	103.7	103.71	103.65
1^1	122.0	122.09	120.49
2^1	130.2	130.29	130.44
3^1	113.3	113.32	114.70
4^1	160.6	160.70	160.71
5^1	113.3	113.32	114.70
6^1	130.2	130.29	130.44
4^1-OMe	55.0	55.15	-
1^2	100.4	100.48	100.34
2^2	73.8	73.89	73.67
3^2	76.0	76.11	75.98
4^2	69.4	69.63	69.36
5^2	76.4	77.23	76.95
6^2	60.5	60.61	61.31

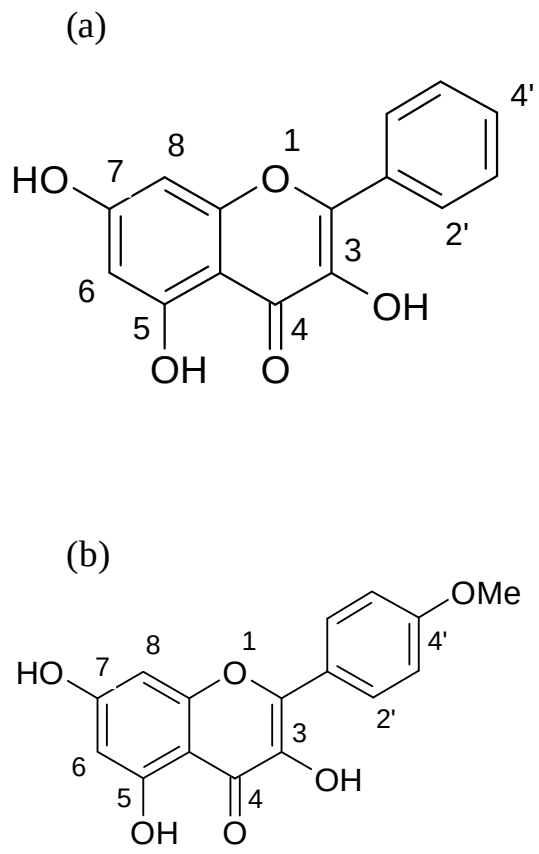

Fig. 01. Structure of isolated compound (a) MKD-1, (b) MKD-2 & (c)MKD-3

of compound, MKD- 2 showed the necessary diagnostic peaks to be identified as a flavonoid derivative with hydroxyl group on C-3, C-5 and C-7, appearing as sharp singlets at δ 9.41, 12.33 and 10.81 ppm, respectively. The H-6 and H-8 occurred as sharp doublet at δ 6.25 and 6.41 ppm, respectively. The B ring signals were easily assigned by consideration of symmetry. The H-2' and H-6' occurred as sharp doublet at δ 8.07 ppm. The H-3' and H-5' appeared as doublet at δ 7.05 ppm, and 4'-OCH_3 occurred as a singlet at δ 3.79 ppm. The 13C experiments of compound 2 gave fourteen peaks. The most downfield shifted peak was 176.0 ppm which was assigned as ketone group (C-4) and most upfield shifted peak was 55.1 ppm which was assigned as ether group (4'-OCH3). Compound MKD-2 exhibited the ^{1}H-NMR and ^{13}C-NMR chemical shifts identical to 3,5,7-trihydroxy-4'-methoxy-flavone (kaempferide) as shown in Table 1 with comparison of its spectral data with previous reports. It's exactly attested by the structure of kaempferide.

3. *Compound MKD-3*

The characteristic bright yellow suggested that compound, MKD-3 is a flavonoid glucoside derivative compound. The IR spectrum of compound, MKD- 3 has shown quite similar to that of compound 2 and important absorptions were attributable to hydroxyl (3650-3200 cm^{-1}) and carbonyl functions (1639 cm^{-1}). Peaks at 2970 and 2825 cm^{-1} were assigned as sp3 C–H, 1639 cm^{-1} as C=O, 1625 and 1500 cm^{-1} conjugated C=C aromatic ring, and 1200 cm^{-1} as C–O as shown in Table 2.

The ^{1}H-NMR and ^{13}C-NMR spectra of compound, MKD-3 showed that all the glucose units had β-linkages to either another glucose or glycone since the constant coupling between the anomeric protons and 22-H was always 7-8 Hz and anomeric carbon resonances were in the δ 100 ppm region. The ^{13}C- NMR spectral data suggested that glucose in compound 3 was attached to the flavonol aglycone at C-3, because of the lack of downfield shift for C-3 and the presence of a hydrogen bonding with the carbonyl group in position 4 at δ 176.84 ppm. Compound MKD-3 exhibited the ^{1}H-NMR and ^{13}C-NMR chemical shifts identical to kaempferide-3-O-β-D-glucoside. A comparison of the 1HNMR and 13C-NMR chemical shifts of compound MKD-3, kaempferide-3-O-β-D-glucoside and kaempferol-3-O-β-D-glucoside is shown in Table 3 and 4. These data indicated that compound MKD-3 is kaempferide-3-O-β-D-glucoside.

Conclusion

The alcoholic crude extract was isolated by column chromatography and preparative thin-layer chromatography to give three pure compounds. The chemical structures were characterized on the basis of NMR spectral analysis, including ^{1}H-NMR and ^{13}C-NMR in comparison with literature values. The structures of three isolated compounds are summarized as follows. Compound, MKD-1 was identified as 3,5,7-trihydroxyflavone. The ^{1}H-NMR and ^{13}C-NMR spectroscopic evidence revealed the presence of the characteristic flavonol group.

Compound, MKD-2 was identified as 3,5,7-trihydroxy-4'-methoxyflavone (kaempferide). The ^{1}H-NMR and ^{13}C-NMR spectra of compound, MKD- 2 were similar to those of compound,MKD- 1, except the presence of extra three CH_3 protons on the B ring.

Compound, MKD-3 was identified as 5,7-dihydroxy-4'-methoxy-3-O-β-D-glucopyranosideflavone (kaempferide-3-O-β-D-glucoside). The ^{1}H-NMR and ^{13}C-NMR spectra of compound, MKD-3 were in agreement with those obtained from the literature.

The findings of study successfully establish the method of isolation of three flavonoids that can be used for the development of novel immunomodulator agents that can overcome side effects.

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