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ISOLATION AND STRUCTURAL ELUCIDATION OF PHYTOCHEMICAL FROM ETHANOLIC EXTRACT OF ROSCEA PROCERA WALL (KAKOLI), AN INGREDIENT OF ASTAVARGA

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ABSTRACT

Roscea procera (Zingiberaceae) commonly known as "Kakoli" from "astavarga" group (group of eight plants). It has wide use in traditional system of indigenous system. The objective of present study was to identify and characterize the bioactive principles from the tubers of Kakoli. In present article, the ethanolic extract of Kakoli was subjected to column chromatography and eluted with solvent mixture of increasing polarity, composed of ethyl acetate, chloroform, acetone and ethanol. The eluted fractions were run in TLC mobile phase with the different solvent ratio. Based on TLC profile the fractions with similar Rf values were pooled together. The structure of the isolated compound was established by physical, chemical and spectroscopic evidences (UV, FT-IR, NMR and MS). The characterizational techniques confirmed that the isolated compound was found to be a flavonoid quercetin.

KEYWORDS: Kakoli, Astavarga, Isolation, flavonoid.

INTRODUCTION

Kakoli (Roscea procera wall.) plants which have been described under "Astavarga" group is selected. Astavarga group have a long history of use in traditional system of indigenous medicine. Astavarga drugs suffer a lot of confusion in Ayurvedic literature in accordance with the identification and authentification.[1]

Kakoli is a perennial herbaceous plant belongs to a family Zingiberaceae. Medicinal plants distributed in Indian Himalayan Region from Himachal Pradesh to Arunachal Pradesh, including Nepal and Bhutan between an altitude of 1800–3000 m asl. It can grow to over 50 cm tall, with wide leaves and a stout pseudostem. The leaf sheaths are pale green or may have a dark reddish-purple tinge. The stem (peduncle) of the flower spike is hidden by the leaf sheaths. The flowers are the largest of any species in the genus. They are usually purple to mauve in colour, although white- and red-flowered forms.[2]

Previous phytochemical studies have demonstrated various secondary metabolites such as alkaloid, glycoside, flavonoid, tannin, saponins and phenolic compounds.[3-7]

As flavonoids are one of the major natural product subgroups in all the plants, it is useful to explore it in kakoli tubers. The search for naturally occurring flavonoids has a great interest in industries as well as scientific research.[8] There was no qualitative and quantitative information on effective isolation on the flavonoids, particularly quercetin content in the tubers of kakoli plant. In this report we have describe the isolation and structural elucidation of flavonoidal compound from ethanolic extract of Kakoli which represent quercetin.

MATERIALS AND METHODS

Plant materials

Marketed rhizomes of Kakoli plant was collected locally, authenticated by Dr. Dongarwar Department of Botany R.T.M. Nagpur University, Campus, Nagpur. A voucher specimen has been deposited in the Herbarium of Department of Botany, with collection no 9480.

Extraction

The dried, coarsely powdered rhizomes of Kakoli plants (500 g each) were extracted with petrolium ether (55-60˚), ethyl acetate (50-55˚), chloroform (50-55˚), acetone (55-60˚), ethanol (60-65˚) successively by soxhletion. The dried marc was cold macerated to obtain hydroalcoholic extract. The extracts were evaporated to dryness in oven (45˚C). The yield of ethyl acetate extract of kakoli, chloroform extract of kakoli, acetone extract of kakoli, ethanol extract of kakoli and hydroalcoholic extract of Kakoli was obtained 2.84 w/w, 3.17 w/w, 1.79 w/w, 2.64 w/w and 2.11 w/w. Chemical constituents of each extract was estimated by qualitative analysis.

Instruments and Chemicals

For elucidation study of isolated bioactive compound of ethanolic extract of kakoli, the IR spectra were recorded on FTIR 8101 A (Schimatzu). ^{1}H and ^{13}C-NMR spectra were run on Bruker Avance II – 400 spectrometer, using the standard pulse program "zgpg30". The mass spectroscopy of isolated compound was carried out using TIC-MS spectrometer.

Column Chromatography[9-11]

10 g of ethanolic extract of kakoli were chromatographed over silica gel column (100-200 mesh) using solvent with increasing polarity. The elution started with 100 % Hexane and then the polarity was increased using mixtures of ethyl acetate, chloroform, acetone and ethanol to obtain fractions for yellow amorphous powder. All the collected fractions were run for TLC. Based on TLC profile fractions with similar Rf values were pooled into same fractions. The substance so obtained was submitted for UV, FTIR, NMR, and Mass spectroscopy studies to elucidate and confirm the structure of isolated bioactive compounds.

RESULTS AND DISCUSSION

Column Chromatography

Ethanol extract (10 g) was subjected to column chromatography on silica gel and eluted with mixture of ethyl acetate, chloroform, acetone and ethanol of increasing polarity, to obtain yellow amorphous powder. About 170 fractions were eluted with different solvents with increasing polarity. Column fraction from 131-145 with ethyl acetate:ethanol (90:10) in TLC mobile phase solvent ratio of chloroform methanol (1:1) showed same Rf value. The fraction showing similar pattern in TLC were mixed together and concentrated in vacuum under reduced pressure. This process was repeated several times by using bulk quantity of samples until the desired amount of isolated compound has been obtained.

Physical Character

Isolated compound was yellow coloured amorphous powder, soluble in methanol and water.

Melting Point

The melting point of isolated compound was recorded by open capillary method using melting point apparatus and it was found to be 312-315°C.

Phytochemical constituent

The isolated compound gave positive reaction for Shinoda test which suggest it to be a flavonoid molecule.

UV Spectra analysis

The UV absorption of isolated compound was determined in methanol over the scanning range of 200-800 nm **(Figure.1)**. The compound showed spectral maxima at 373 nm and 256 nm and λmax was found to be 373 nm. The typical UV- Vis spectra of flavonoid include two absorbance band. Band A lies in the 310-350 nm range for flavones, while for flavonoles it is between 350-385 nm. Band B, found in the 250-290 range.[12]

Thus the UV spectrum showed that the compound may belongs to flavonol category of flavonoid. Consequently, these two sub groups cannot be distinguished by simple UV- Vis analysis. Flavonols showed maximum absorbance at nonspecific wavelengths between 270 and 290 nm, at which many phenolics absorb, thus not allowing their selective detection.

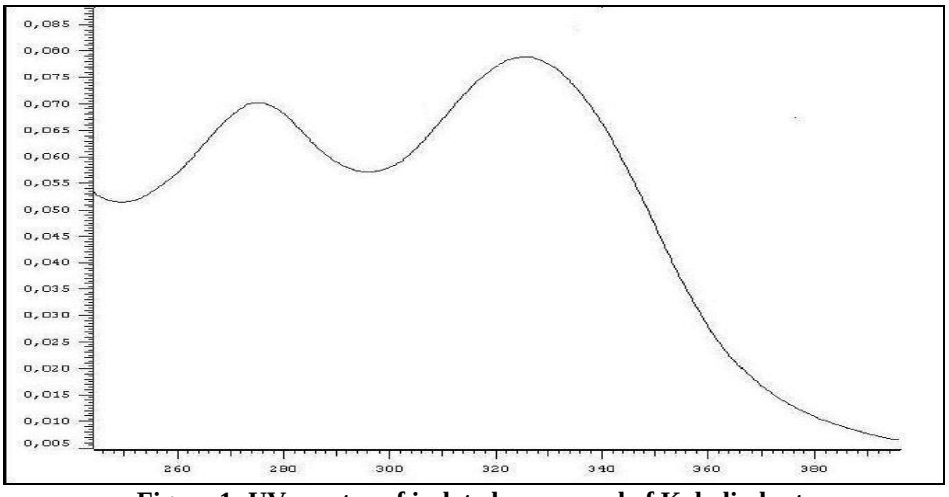


Figure 1: UV spectra of isolated compound of Kakoli plant.

Fourier transforms infrared (FT-IR) spectrum analysis

The FT-IR spectrum of isolated compound was shown in **Figure 2** and their corresponding characteristic peak positions were listed in **Table 1**. The broad absorption peak at around 3409 cm^{-1} was assigned to the OH stretching vibration of phenol. C=O aryl ketonic stretching vibrations are observed at 1665 cm^{-1}. The absorption peaks positioned at 1609 cm^{-1}, 1522 cm^{-1} and 1458 cm^{-1} are assigned to the C---C, C=O and C=C aromatic stretching vibrations respectively. OH bending vibrations of phenols were observed at 1382 cm^{-1}. The absorption peak at 1320 cm^{-1} and the peaks at the lower frequencies between 1014 cm^{-1} and 493 cm^{-1} were assigned to the C-H bending vibrations of aromatic hydrocarbons. C-O stretching vibrations of aryl ether and phenols were observed at 1264 cm^{-1} and 1201 cm^{-1} respectively. C-CO-C stretching and bending vibrations of ketones were observed at 1170 cm^{-1}.

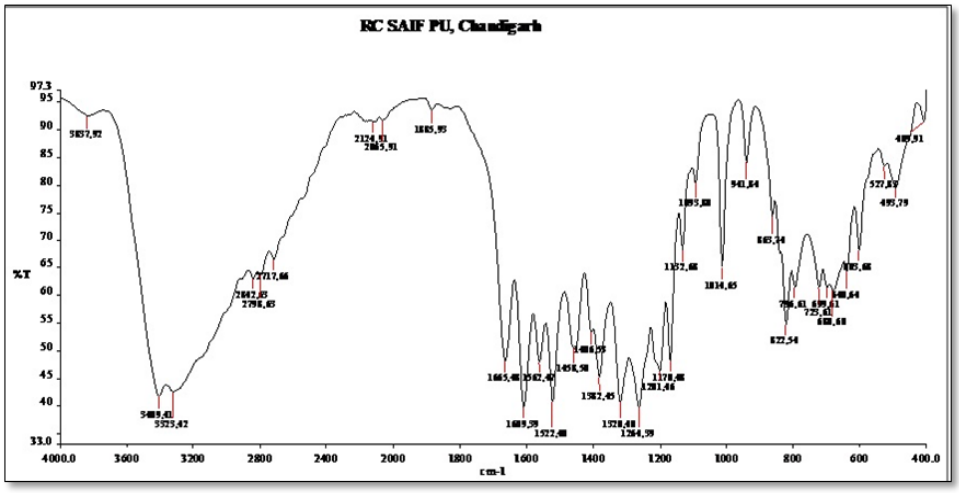


Figure 2: FT-IR spectra of isolated compound of Kakoli plant.

Table 1: Infra red band frequencies of isolated compound of kakoli plant and its correlation to structural assignment.

Sr. No.	Wave number (cm^{-1})	Inter-atomic bond
1	3409.41	O-H stretch; phenol
2	1665.48	C=O aryl ketonic stretch
3	1609.39	C---C Aromatic ring stretch
4	1522.40	C=O Aromatic stretch
5	1458.50	C=C Aromatic stretch
6	1382.45	O-H bending of Phenol
7	1320.40	C-H in Aromatic hydrocarbon
8	1264.39	C-O stretch of aryl ether
9	1201.46	C-O of Phenol
10	1170.48	C-CO-C stretch and bending in ketone
11	1014.65, 941.84, 822.54, 723.61, 640.64, 493.79	C-H bending of aromatic hydrocarbon

NMR spectrum of isolated compound

The structure of the isolated compound of kakoli plant was characterized by spectroscopic techniques like ^{1}H and ^{13}C NMR spectrum. The sample were dissolved in DMSO. NMR studies were carried out to confirm the positions of proton and carbon binding sites. The ^{1}H-NMR spectrum of the isolated compound showed aromatic hydrogen groups from 6.19-7.67 ppm and phenolic-OH groups from 9.36-12.49 ppm respectively (**Table 2 and Figure 3**). The 13C-NMR spectrum showed carbonyl group at 175.8 ppm and aromatic carbon group from 93.31-163.84 ppm was shown in (**Table 2 and Figure 4**).

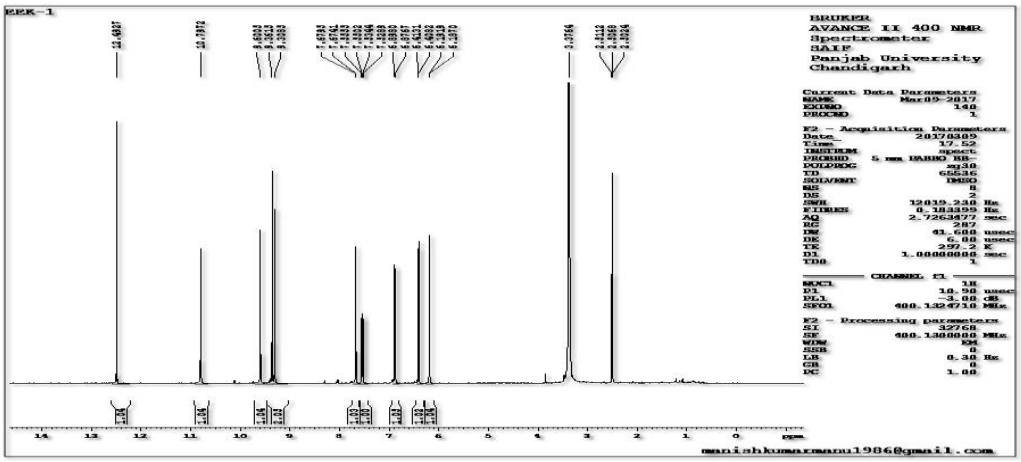


Figure 3: ^{1}H NMR spectra of isolated compound of Kakoli plant.

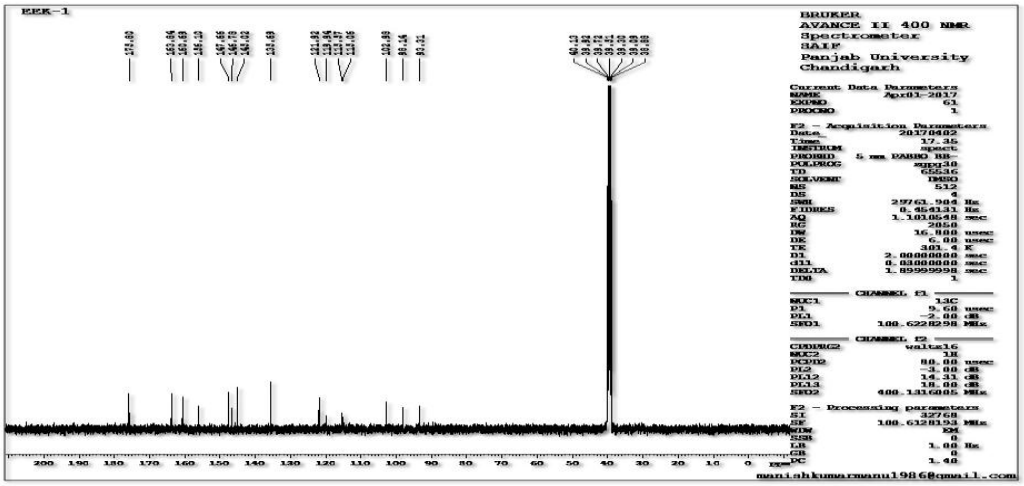


Figure 4: ^{13}C NMR spectra of isolated compound of Kakoli plant.

Table 2: ^{1}H and ^{13}C NMR Chemical shift of isolated compound of kakoli plant and its correlation to structural assignments.

^{1}H NMR spectrum of isolated compound		^{13}C NMR spectrum of isolated compound	
Chemical Shift (ppm)	**Inter-atomic bond**	**Chemical Shift (ppm)**	**Inter-atomic bond**
6.19	Ar-H	93.31	Ar-C
6.41	Ar-H	98.14	Ar-C
6.89	Ar-H	102.98	Ar-C
7.53	Ar-H	115.06	Ar-C
7.67	Ar-H	115.57	Ar-C
9.36	Ar-OH	119.94	Ar-C
9.6	Ar-OH	121.92	Ar-C
10.79	Ar-OH	135.69	Ar-C
12.49	Ar-OH	145.02	Ar-C
		146.78	Ar-C
		147.66	Ar-C
		156.10	Ar-C
		160.69	Ar-C
		163.84	Ar-C
		175.80	Ar-C=O

Mass spectroscopy of isolated compound

The MS spectra of isolated compound was performed in positive ion mode and molecular ion peak was observed at m/z 302.95. thus the mass spectra of isolated compound suggested that its molecular mass is 302.95 (M.F. $C_{15}H_{10}O_7$) having characteristic fragments observed at m/z: 325, 345.95, 349.05 (**Figure 5**).

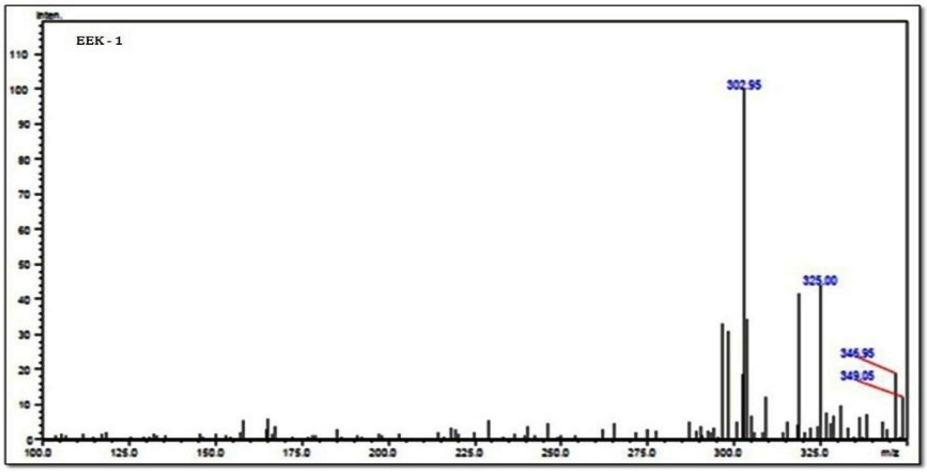


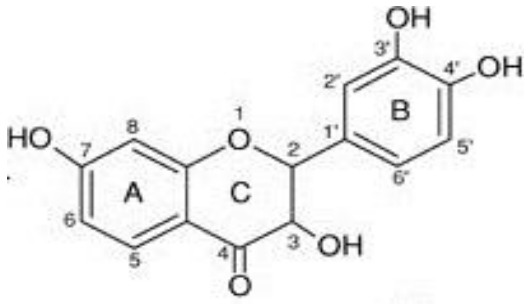
Figure 5: Mass spectra of isolated compound of Kakoli plant.

Inference

From the above spectroscopic study, the isolated compound of kakoli plant was found to be aromatic which are reflected in IR peaks. The mass spectra of the isolated compound has molecular ion peak at 302.95 suggesting the molecular weight to be 302. Thus from the IR, NMR and mass spectroscopy the compound was ascertain as 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one.

The phytochemical screening of isolated compound suggested it to be flavonoidal molecule. The UV-Vis spectrum obtained was also in agreement with published UV spectrum of Quercetin. The melting point of compound was same as that of Quercetin. From the above spectral data the compound had resemblance to Quercetin.

Hence molecular formula of compound was $C_{15}H_{10}O_7$ and the molecular structure was ascertained as 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one.



Molecular Weight: 302.95/Molecular Formula: $C_{15}H_{10}O_7$.

IUPAC Name: 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4one.

CONCLUSION

In conclusion, the compound isolated from the ethanolic extract of Kakoli demonstrated positive tests for flavonoids. From all the furnished physical, chemical and spectral evidences like UV, FT-IR, NMR and Mass spectroscopy, the isolated crystalline compound was confirmed as quercetin.

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