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Isolation of Two Phenolic Compounds from Ethanol Extract of Leaves of *Corchorus fascicularis* Lam.

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Abstract: The present study aim was to identify and characterize the active principles from the leaves of *Corchorus fascicularis* Lam. For isolation of compounds, the dried leaves powder of *Corchorus fascicularis* Lam. was subjected to cold maceration with ethanol as solvent and subjected to column chromatography. Two compounds were isolated and purified by methanol. Mass spectrum of EEC-1 and EEC-2 showed a parent molecular ion peak at m/z 302 and 290 which corresponds to molecular formula $C_{15}H_{14}O_7$ and $C_{15}H_{14}O_6$. From physical, chemical and spectral characteristics EEC-1 and EEC-2 were concluded as Quercetin and Catechin.

Key words: Quercetin, catechin, cold maceration, ethanol extracts, *Corchorus fascicularis*

INTRODUCTION

Corchorus fascicularis L belonging to family Tiliaceae is an annual herb found in throughout India and also many tropical countries. The leaves are tasty and sourly shown activity of Laxative, stimulant, tonic, aphrodisiac and also destroy "tridosha"; the seeds are hot with a sharp taste, removes tumors, pain, stomach troubles, skin diseases, scabies and useful in discharging ulcers (Kirtikar and Basu, 1996). A new cardiac glycoside strophanthidin-3-β-D-bolvinosido-β-D-glucoside corchoroside A present in seeds and also shows components of glycoside mixture-olitoriside, ursolic acid, oxocorosin and corosolic acid isolated from roots; Glycoside 2α, 3β, 20β, Urs-12 en-23β, 28-dioic acid 2, 3-diacetate showed analgesic results in chemical tests and showed anti pyretic activity (Sing and Panda, 2005). The pent acyclic betulinic acid and steroid were isolated from Whole plant of *C. fascicularis* Lam. (Khan *et al*., 2006). The powder of *Corchorus fascicularis* Lam. were used for tonic (Patil, 2003). *Corchorus fascicularis* L. reported for physiological activity (Hossen *et al*., 2008). Glycosides were isolated from *Corchorus fascicularis* L. (Tariq *et al*., 1973). *Corchorus fascicularis* L. used for astringent, blood purifier, concoctive, mucilaginous, resolvent and restorative (Nadkarni, 2005). *Corchorus fascicularis* L. Leaves shown antimicrobial activity (Rajput and Rajput, 2011b). Leaves of *Corchorus fascicularis* L. were shown presence of phytoconstituents (Rajput and Rajput, 2011a). The purpose of this study was to identify and characterized the active principles from the leaves of the *Corchorus fascicularis* Lam.

MATERIALS AND METHODS

Preparation of plant material: The leaves of plant *Corchorus fascicularis* L. were collected from village Tande of Shirpur in Dhule district (MS); India in the month of July 2009. The plant was taxonomically identified by Professor Dr. L. K. Kshirsagar, Taxonomist, Department of Botany, S.S.V.P.S's L.K. Dr. Ghogrey Science College, Dhule, North Maharashtra University, Jalgaon. The dried leaves powder (3 kg) was subjected for extraction with ethanol by cold maceration at room temperature (Harborne, 1998).

Isolation and purification of compounds: A small quantity of ethanol extract was dissolved in ethanol and this solution was spotted on TLC Plates. Silica gel 60F254 precoated plates (Merck) were used for TLC. The spots were detected by spraying 70% Ethanolic-H_2SO_4 reagent followed by heating. All chemicals and reagents used for TLC were of analytical grade. Then the TLC plates were run by specific solvent system and were viewed individually in iodine chamber and with the 70% Ethanolic-H_2SO_4 spraying reagent. Through several pilot experiments, it was found that the compounds of chloroform extract fraction were separated by solvent system of chloroform, methanol and ethyl acetate in the proportion of 7:2:1. Seven grams ethanol extract was subjected to column chromatography on silica gel (60-120 mesh) with gradient elution using chloroform:methanol:ethyl acetate (Stahl, 1969).

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Two fractions were found homogeneous on TLC plate by using Chloroform: ethyl acetate (9.2:0.8), Petroleum ether:chloroform (9.5:0.5), toulene:ethyl acetate:metahnol (7:2:1) solvent systems. These fractions were crystallized (Bahl and Bahl, 1992) and named as EEC-1 (ethanol extract compound-1) and EEC-2 (ethanol extract compound-2), respectively.

Test for flavonoids

Shinoda test (magnesium hydrochloride reduction test): A few crystals of EEC-1 and EEC-2 were dissolved in Ethanol and a magnesium ribbon and drop wise concentrated Hydrochloric acid drop wise added to the solution, for both EEC-1 and EEC-2 formed a crimson red color after few minutes it converted to blue color indicating presence of flavonoids (Harborne, 1998).

Zinc-hydrochloride reduction test: A few crystals of EEC-1 and EEC-2 were dissolved in Ethanol; then mixture of Zinc dust and conc. Hydrochloric acid solution was added, both EEC-1 and EEC-2 developed red color after few minutes indicating presence of flavonoids (Harborne, 1998).

Alkaline reagent test: A few crystals of EEC-1 and EEC-2 were dissolved in ethanol. In this solution few drops of Sodium hydroxide solution were added, both EEC-1 and EEC-2 formed an intense yellow color which turns to colorless on addition of few drops of dilute acetic acid indicating the presence of flavonoids.

Spectroscopic characterization: Different spectroscopic methods were used to elucidate the structure of EEC-1 and EEC-2. Among the spectroscopic technique IR, ^{1}H-NMR, ^{13}C-NMR and LC-MS were carried out. The infrared spectrum was recorded on FT-IR Spectrum one (Perkin Elmer, USA), ^{1}H-NMR spectra were recorded on a Varian-400 MH_Z NMR spectrometer, Mercury Plus (Switzerland), ^{13}C-NMR spectra were recorded on a Varian-400 MH_Z NMR spectrometer Mercury Plus (Switzerland) at Wockhardt R and D Ltd., Aurangabad, India. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded using $CDC1_3$, as solvent with Tetramethyl silane (TMS) as an internal standard. Mass spectrum was recorded at high resolution on a mass spectrometer (Perkin Elmer Autosystem XL with Turbomass) at Research and Development centre; the data are given in m/z values. Elemental analysis was recorded on Elementar instrument model Vario Micro (Germany) using oxygen and helium as combustion and carrier gases, respectively at a temperature of 1150°C at Wockhardt Research and Development Centre, Aurangabad, India.

RESULTS

From the positive tests for Flavonoids given by the EEC-1 and EEC-2, they were assumed to be flavonoids. The melting point of EEC-1 and EEC-2 were 140°C and 273°C, respectively. The UV λ_{max} value of EEC-1 and EEC-2 was formed at 251 and 276 nm, respectively. Mass spectrum of EEC-1 and EEC-2 showed a parent molecular ion peak at 302 and 290, respectively, which corresponds to the molecular formula $C_{15}H_{10}O_7$ (Fig. 1) and $C_{15}H_{14}O_6$ (Fig. 2).

In the IR spectrum of EEC-1 and EEC-2, an intensely broad band at 3368 and 3220 cm^{-1} showed presence of OH stretching and in the ^{1}H-NMR spectrum of EEC-1 it was seen that H-3 proton appeared at δ12.49 as hydroxyl proton with carbonyl system. The signal at 10.74 strong singlet for H-5 due to aromatic hydroxyl group and downfield due to near electronic withdrawing group. In ^{13}C-NMR spectrum, the signal corresponding to the ketonic carbonyl group C-4 appeared at δ176.51. The signals at δ164.56, δ161.40, δ148.37, δ145.73, δ136.40 for five hydroxyl groups (Table 1).

The ^{1}H-NMR data of EEC-2 showed that the signal at δ9.14 due to aromatic phenolic groups. The signals at δ6.70, δ6.58, δ6.56, δ5.86, δ5.66 due to different five aromatic protons. In the ^{13}C-NMR spectra, signals appeared at δ28.52, 66.98, 81.64 due to C-4, C-3, C-2 carbons, respectively and other aromatic carbons showed

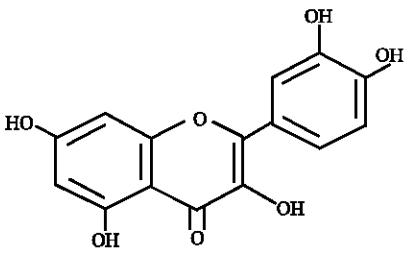

Fig. 1: Chemical structure of EEC-1 (quercetin)

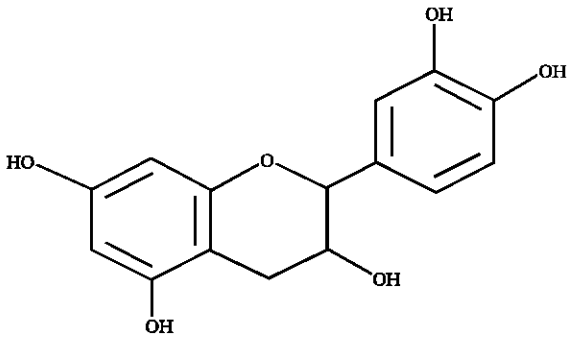

Fig. 2: Chemical structure of EEC-2 (catechin)

Table 1: Spectroscopic data of EEC-1 (quercetin)

Spectroscopic technique	Data
CHN analysis	C = 59.56 %, H = 3.518%
UV λ max	256 nm
IR: ($CHCl_3$):	3368, 3082, 2840, 1760, 1673,1522,1457, 1430, 1365, 1096, 1014, 716, 691 cm^{-1}
LCMS	302
^{1}H-NMR ($CDCl_3$)	δ12.49 (ss, OH C-5), 10.74 (ss, OH C-7), 9.55 (s, OH C3'), 9.33 (s, OHC-4'), 9.27 (s, OH C-3), 7.66 (d, 1H H-2'), 7.53 (dd, 1H, H-6'),6.87 (d, 1H, H-5'), 6.38 (d, 1H, H-8), 6.16 (d, 1H, H-6)
^{13}C-NMR ($CDCl_3$)	δ176.5 (C-4), δ164.56 (C-7), δ161.40 (C-5), δ156.81 (C-9), δ148.37 (C-4'), δ147.48 (C-2), δ145.73 (C-3'), δ136.40 (C-3), δ122.63 (C-1'), δ120.65 (C-6'), δ166.28 (C-5'), δ115.74 (C-2'), δ103.69 (C-10), δ98.85 (C-6), δ94.02 (C-8)

IR: Infrared spectroscopy, LCMS: Liquid chromatography mass spectroscopy, ^{1}H-NMR: Proton nuclear magnetic spectroscopy, ^{13}C-NMR: Carbon nuclear magnetic spectroscopy, UV: Ultraviolet spectroscopy

Table 2: Spectroscopic data of EEC-2 (catechin)

Spectroscopic technique	Data
CHN analysis	C = 61.13%, H = 5.080%
UV λ max	251 nm
IR: ($CHCl_3$):	3240, 2853, 1629, 1522, 1473,1376, 1237, 1080, 1030, 732, 674
LCMS	290
^{1}H-NMR ($CDCl_3$)	δ9.14 (m, phenolic protons), δ6.70 (d, 2'H), δ6.58 (d, 5' H), (chemical shift in δ ppm) δ6.56 (dd, 6'H), δ5.86 (d, 6H), δ5.66 (d, 8H), δ4.46 (d, H2), δ3.79 (ddd, 3H), δ2.64 (dd, 4 H), δ2.33 (dd, 4' H)
^{13}C-NMR ($CDCl_3$)	δ157.12 (C-9), δ156.84 (C-7), δ156.02 (C-5), δ145.51 (C-3'), δ144.01 (C-4'), δ131.27 (C-1'), δ119.10 (C-6'), δ115.75 (C-5'), δ115.19 (C-2'), δ99.73 (C-10), δ95.79 (C-6), δ94.52 (C-8), δ81.64 (C-2), δ66.98 (C-3), δ28.52 (C-4)

IR: Infrared spectroscopy, LCMS: Liquid chromatography mass spectroscopy, ^{1}H-NMR: Proton nuclear magnetic spectroscopy, ^{13}C-NMR: Carbon nuclear magnetic spectroscopy, UV: Ultraviolet spectroscopy

peaks at δ94.52, δ95.79, δ99.73, δ115.19, δ115.75, δ119.10, δ131.27, δ144.01, δ145.51, δ156.02, δ156.84, δ157.12 (Table 2). From above observation EEC-1 and EEC-2 were found to be Quercetin and Catechin.

DISCUSSION

In IR spectrum of EEC-1, a very intensely broad band at 3368.18 cm^{-1} and moderately intense band at 1365 and 691 cm^{-1} were observed for the O-H bond vibrations of hydroxyl group. The corresponding aromatic C = C vibrations was shown around 1618 cm^{-1} as weakly intense band. The stretching and bending vibrations of methylene part were noticed by the intense band at 2840 cm^{-1} and medium intensity band at 1457 cm^{-1}. The corresponding carbonyl function stretching vibrations at 1760 cm^{-1}. The very weak band at 716 cm^{-1} was attributed to the rocking movement of methylenic part. The corresponding C-C vibration was shown as weak intense band at 1096 cm^{-1}.

The ^{1}H-NMR data of EEC-1, H-3 proton appeared at δ12.49 as hydroxyl proton with carbonyl system. The strong singlet at δ10.74 was assigned to H-5 which could be due to aromatic hydroxyl group and down field value due to presence of electron withdrawing carbonyl group. The signal at δ9.55, δ9.32 and δ9.27 due to three aromatic hydroxyl groups. Five Aromatic protons shows signal at δ7.65, δ7.52, δ6.87, δ6.38 and δ6.16. The observed signals in NMR spectra were in good agreement with the authentic Quercetin (Aderogba *et al*., 2006).

In ^{13}C-NMR spectrum, the signal corresponding to the ketonic carbonyl group C-4 appeared at δ176.51. The signals at δ164.56, δ161.40, δ148.37, δ145.73, δ136.40 for five hydroxyl groups. The ^{13}C-NMR spectral data were similar to the ones reported for Quercetin (Li *et al*., 2008), (Table 1).

Similarly in IR spectrum of EEC-2, a very intensely broad band at 3240.85 cm^{-1} and moderately intense band at 1376 cm^{-1} and 674 cm^{-1} were observed for the O-H bond vibrations which can be attributed to of hydroxyl groups. The corresponding aromatic C = C vibrations was shown around 1629 cm^{-1} as weakly intense band. The stretching and bending vibrations of methylene part were noticed by the intense band 2853 cm^{-1} and medium intensity band at 1473 cm^{-1}. The very weak band at 732 cm^{-1} was attributed to the rocking movement of methylenic part. The corresponding C-C vibration was shown as weak intense band at 1080 cm^{-1}. Asymmetric C-O-C stretching showed intense band at 1237 cm^{-1}.

The ^{1}H-NMR data of EEC-2 showed that the signal at δ9.14 due to aromatic phenolic groups. The signals at δ6.70, δ6.58, δ6.56, δ5.86, δ5.66 due to different five aromatic protons. The signal at δ4.46 was assigned to alcoholic proton which was in good agreement with reported value (Jung *et al*., 2012).

In the ^{13}C-NMR spectra, signals appeared at δ28.52, 66.98, 81.64 due to C-4, C-3, C-2 carbons, respectively and others aromatic carbons showed peaks at δ94.52, 95.79, 99.73, 115.19, 115.75, 119.10, 131.27, 144.01, 145.51, 156.02, 156.84, 157.12. These data were in good agreement with the reported Catechin (Falah *et al*., 2008; Hye *et al*., 2009).

The spectral data of compound EEC-1 and EEC-2 for quercetin, catechin, respectively are similar for reported spectral data by Harborne and Mabry (1982) and Markham (1982).

CONCLUSION

The isolation by cold maceration method resulted into two compounds EEC-1 and EEC-2. As per our aim these compounds were characterized by using physical, chemical and modern spectral analysis. In future the active medicaments can be studied further for their pharmacological and biological activities. The leaves of these plants may show good anti-oxidant activity.

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