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Isolation of Stigmasterol and β-Sitosterol from Chloroform Extract of Leaves of *Corchorus fascicularis* Lam.

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ABSTRACT

The present study aimed 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 chloroform as solvent and subjected to chromatography. Two compounds were isolated and purified by chloroform. Mass spectrum of CEC-1 and CEC-2 showed a parent molecular ion peak at m/z 412 which correspond to molecular formula $C_{29}H_{48}O$ and 414 corresponds to $C_{29}H_{50}O$. In ^{1}H-NMR spectrum of CEC-1, H-3 proton appeared as a triplet of a double doublet (tdd) at δ 3.51 and H-6 olefinic proton showed a multiplet at δ 5.14. Two olefinic protons appeared downfield at δ 4.16 (m) and δ 4.14 (m), six methyl protons also appeared at δ 1.21, δ 1.17, δ 1.03, δ 0.99, δ 0.97 and δ 0.90 (3H each, s, CH_3).The ^{1}H-NMR data of CEC-2 it was seen that H-3 proton appeared at δ 3.52 as a triplet of a doublet of doublet and H-6 olefinic proton showed a multiplet at δ 5.35. Moreover, six methyl protons appeared at δ 1.26, δ 1.17, δ 1.03, δ 1.00, δ 0.91 and δ 0.90 for H-19, H-18, H-26, H-27, H-21, H-29, respectively for methyl group. From physical, chemical and spectral characteristics CEC-1 and CEC-2 were concluded as Stigmasterol and β-Sitosterol.

Key words: Stigmasterol, β-sitosterol, *Corchorus fascicularis* Lam., column chromatography, chloroform extract, cold maceration

INTRODUCTION

Corchorus fascicularis Lam. is an annual herb found throughout India and also in many tropical countries (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. Urosolic acid, oxocorosin and corosolic acid isolated from roots; Glycoside 2α, 3 β, 20 β, Urs-12 en-23 β, 28-dioic acid 2, 3-diacetate showed analgesic results in chemical tests and showed anti pyretic activity (Sing and Panda, 2005). The leaves are tasty and sourly show 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 and scabies. It was useful in discharging ulcers (Kirtikar and Basu, 1996). The powder of *Corchorus fascicularis* Lam. were used for tonic (Patil, 2003). This plant is used for treatment of Dysentery, Diarrhoea and Gynecological problems (Singh *et al*., 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, 2011a). Leaves of *Corchorus fascicularis* L. were reported for presence of steroids (Rajput and Rajput, 2011b). In Ayurvedic system of medicines this plant has a large demand due to its uses in the treatment of many chronic and acute diseases and disorders. In this study, we have isolated two steroids namely stigmasterol and **β**-Sitosterol using column chromatography and are identified by ^{1}H-NMR, ^{13}C-NMR spectroscopic methods.

MATERIALS AND METHODS

Preparation of plant material: The leaves of plant *Corchorus fascicularis* L. were collected from village Tande of Shirpur tehasil 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 extraction with Chloroform by cold maceration (Harborne, 1998) and evaporation of solvent 5 gm extract was found.

Isolation and purification of compounds: A small quantity of chloroform extract was dissolved in chloroform and this solution was spotted on Thin Layer Chromatography (TLC) plates. Then the TLC plates were run by specific solvent system and were view individually in iodine chamber and with the 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 and ethyl acetate in the proportion of 5:5. The chloroform extract 5 gm was subjected to column chromatography on silica gel (60-120 mesh) with gradient elution using Chloroform: Ethyl acetate (Stahl, 1969).

Two fractions were found homogeneous on TLC plate by using (Chloroform: Ethyl acetate) (5:5), (petroleum ether: ethyl acetate) (9.0:1.0), (benzene:chloroform) (9.5:0.5), (chloroform:ethyl acetate) (9.0:1.0), (chloroform:ethyl acetate) (8.0:2.0) solvent systems. These fractions were crystallized (Bahl and Bahl, 1992) and named as CEC-1 (chloroform extract compound-1) and CEC-2 (chloroform extract compound-2), respectively.

Test for alcohol: Four gram of ceric ammonium nitrate was dissolved in 10 mL of 2 N HNO_3, on mild heating. A few crystals of CEC-1 and CEC-2 were dissolved in 0.5 mL of dioxane. The solution was added to 0.5 mL of ceric ammonium nitrate reagent and diluted to 1 mL with dioxane and shaken well. Both CEC-1 and CEC-2 developed yellow to red color indicating the presence of an alcoholic hydroxyl group (Harborne, 1998).

Test for steroid

Salkowski reaction: A few crystals of CEC-1 and CEC-2 were dissolved in chloroform and a few drops of concentrated sulphuric acid were added to the solution, both CEC-1 and CEC-2 formed a reddish color in the upper chloroform layer (Harborne, 1998) indicating presence of steroids.

Liebermann-Burchard reaction: A few crystals of CEC-1 and CEC-2 were dissolved in chloroform and few drops of concentrated sulfuric acid were added to it followed by the addition of 2-3 drops of acetic anhydride. In this case both CEC-1 and CEC-2 turned to violet blue and finally formed green color which indicates the presence of steroids (Harborne, 1998).

Spectroscopic characterization: Different spectroscopic methods were used to elucidate the structure of CEC-1 and CEC-2. Among the spectroscopic technique IR, ^{1}H-NMR, ^{13}C-NMR and

LC-MS were carried out. The infrared spectrum was recorded on FTIR 8400 (Shimadzu), ^{1}H-NMR spectra were recorded on a Varian-400 MH_Z NMR spectrometer (Shimadzu), ^{13}C-NMR spectra were recorded on a Varian-400 MH_Z NMR spectrometer (Shimadzu) at Wokhardt R and D Ltd., Aurangabad, India. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded using $CDC1_3$ as solvent with Tetramethylsilane (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 Cube using oxygen and helium as combustion and carrier gases respectively at a temperature of 1150°C Wockhardt Research and Development Centre, Aurangabad, India.

RESULTS

From the positive tests for steroids and alcohols given by the CEC-1 and CEC-2, they were assumed to be sterol. The melting point of CEC-1 and CEC-2 were 167 and 138°C, respectively. The UV λ_{max} value of CEC-1 and CEC-2 was found at 256 and 251 nm, respectively. Mass spectrum of CEC-1 and CEC-2 showed a parent molecular ion peak at 412 and 414, respectively which corresponds to the molecular formula $C_{29}H_{48}O$ (Fig. 1) and $C_{29}H_{50}O$ (Fig. 2).

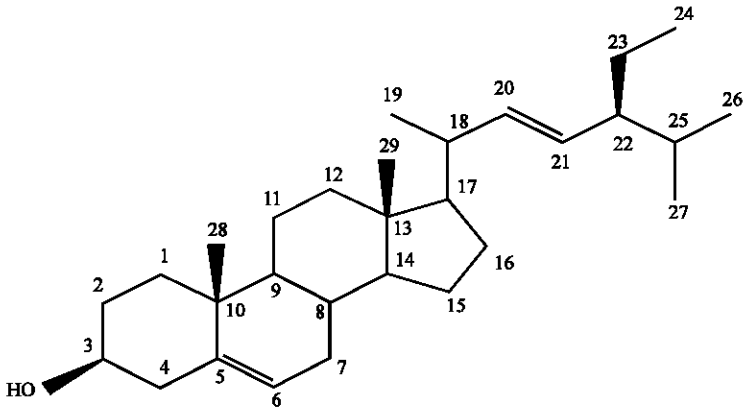

Fig. 1: Chemical structure of CEC-1 (stigmasterol)

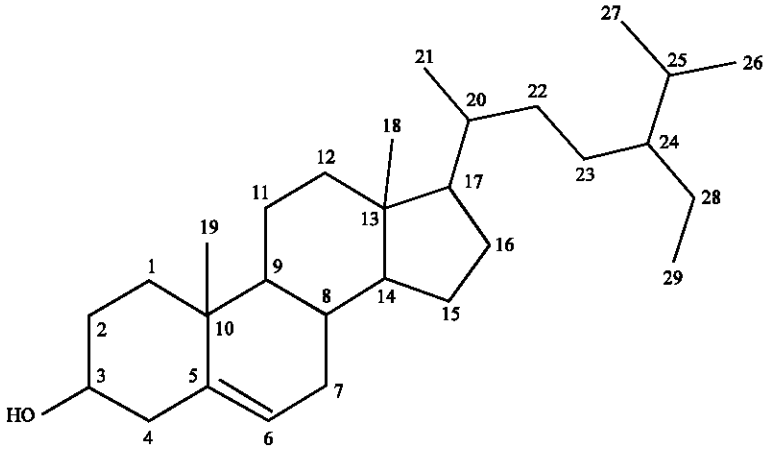

Fig. 2: Chemical structure of CEC-2 (β-sitosterol)

Table 1: Spectroscopic data of CEC-1 (Stigmasterol)

Spectroscopic technique	Data
CHN analysis	Found: C = 84.32%, H = 11.77%, Calculated: C = 84.34%, H = 11.72%
UV (λ_{max})	256 nm
IR: ($CHCl_3$)	3335, 2934, 2866, 1667, 1459, 1193, 1089, 1036, 777, 667 cm^{-1}
LC-MS	412, 144, 198, 229, 243, 264, 265, 289, 346, 382, 391, 396
^{1}H-NMR ($CDCl_3$)	δ7.30 (s, OH-3), 5.14 (m, 1H, H-6), 4.60 (s, 1H, H-22), 4.23 (s, 1H, H-23), 3.51 (tdd, H-3, H-3), 1.21 (s, 3H, H-19), 1.17 (s, 3H, H-28), 1.03 (s, 3H, H-27), 0.99 (s, 3H, H-26), 0.97 (s, 3H, H-24), 0.90 (s, 3H, H-29)
^{13}C-NMR ($CDCl_3$)	δ 140.9 (C-22), 138.53 (C-5), 129.50 (C-23), 121.89 (C-6), 71.9 (C-3), 57.1 (C-4), 56.2 (C-5), 51.4 (C-24), 50.40 (C-17), 42.5 (C-9), 42.4 (C-13), 40.7 (C-10), 39.9 (C-10), 37.5 (C-20), 32.9 (C-25), 31.9 (C-21), 29.1 (C-23), 25.6 (C-12), 24.6 (C-11), 21.5 (C-25), 21.3 (C-26), 19.2 (C-29), 12.3 (C-27)

Table 2: Spectroscopic data of CEC-2 (β-Sitosterol)

Spectroscopic technique	Data
CHN analysis	Found: C = 84.15%, H = 12.23%, Calculated: C = 83.99%, H = 12.15%
UV λ_{max}	251 nm
IR: ($CHCl_3$)	3331, 2959, 1654, 1464, 1382, 1062, 800
GC-MS	414, 396, 381, 329, 303, 289, 273, 255, 231, 213, 199, 173, 159, 145, 119, 95, 81, 69, 55
^{1}H-NMR ($CDCl_3$)	δ 7.35 (s, O-H), 5.35 (m, 1H, H-6), 3.52 (tdd, 1H, H-3),1.26 (s, 3H, H-19), 1.17 (s, 3H, H-18), 1.03 (s, 3H, H- 26), 1.00 (s, 3H, H-27), 0.91(s, 3H, H-21), 0.90 (s, 3H, H-29)
^{13}C-NMR ($CDCl_3$)	δ 140.9 (C-5), 121.9 (C-6), 71.5 (C-3), 56.2 (C-4), 50.3 (C-17), 46.1 (C-14), 42.5 (C-13), 40.0 (C-10), 37.5 (C-10), 33.4 (C-20), 31.7 (C-25, C-26), 28.1 (C-23), 25.1 (C-12), 21.0 (C-18, C-19), 19.1 (C-21), 15.1 (C-29), 12.8 (C-27)

In the IR spectrum of CEC-1, an intensely broad band at 3335 and 3431 cm^{-1} showed presence of O-H stretching and in the ^{1}H-NMR spectrum of CEC-1, In ^{1}H-NMR spectrum of CEC-1, H-3 proton appeared as a triplet of a double doublet (tdd) at **δ** 3.51 and H-6 olefinic proton showed a multiplet at **δ** 5.14. Two olefinic protons appeared downfield at **δ** 4.16 (m) and **δ** 4.14 (m), six methyl protons also appeared at **δ** 1.21, **δ** 1.17, **δ** 1.03, **δ** 0.99, **δ** 0.97 and **δ** 0.90 (3H each, s, CH_3) (Table 1).

The ^{1}H-NMR data of CEC-2 it was seen that H-3 proton appeared at **δ** 3.52 as a triplet of a doublet of doublet and H-6 olefinic proton showed a multiplet at **δ** 5.35. Moreover, Six methyl proton appeared at **δ** 1.26, **δ** 1.17, **δ** 1.03, **δ** 1.00, **δ** 0.91 and **δ** 0.90 (3H each, s, CH_3) for H-19, H-18, H-26, H-27, H-21, H-29, respectively for methyl group (Table 2).

The ^{1}H-NMR and ^{13}C-NMR data of CEC-1 and CEC-2 were quite similar with the data in the literature of stigmasterol and **β-** sitosterol, respectively. From above observation CEC-1 and CEC-2 were found to be stigmasterol and **β**-sitosterol.

DISCUSSION

In IR spectrum of CEC-1, a very intensely broad band at 3335 cm^{-1} and moderately intense band at 1193 and 667 cm^{-1} were observed for the O-H bond vibrations of hydroxyl group. The out of plane C-H vibrations of the unsaturated part was observed at 879 cm^{-1}. The corresponding C = C vibrations was shown around 1667 cm^{-1} as weakly intense band. The stretching and bending vibrations of methyl part were noticed by the intense band at 2934 cm^{-1}. The vibration of the methylene part was shown by the band at 2866 cm^{-1} and the medium band at 1459 cm^{-1}. The moderately intense band at 777 cm^{-1} was attributed to the rocking movement of methylene part. The corresponding C-C vibration was shown as weak intense band at 1036 cm^{-1}. These assignments were good agreements with reported values (Muhit *et al.*, 2010; Ian *et al.*, 1976).

In ^{1}H-NMR spectrum of CEC-1, H-3 proton appeared as a triplet of a double doublet (tdd) at δ 3.51 (J = 4.5 and 1.0 MH_Z) and H-6 olefinic proton showed a multiplet at δ 5.14. Two olefinic protons appeared downfield at δ 4.16 (m) and δ 4.14 (m) which were identical with the chemical shift of H-22 and H-23, respectively of stigmasterol (Habib *et al.*, 2007; Jain *et al.*, 2009). Six methyl protons also appeared at δ 1.21, δ 1.17, δ 1.03, δ 0.99, δ 0.97 and δ 0.90 (3H each, s, CH_3). These assignments were good agreements with reported values (Jain and Bari, 2010; Hartati *et al.*, 2008; Li *et al.*, 2006).

In IR spectrum of CEC-2, a very intensely broad band at 3331 cm^{-1} and moderately intense band was observed for the O-H bond vibrations of hydroxyl group. The corresponding C = C vibrations was shown around 1654 cm^{-1} as weakly intense band. The stretching and bending vibrations of methyl part were noticed by the intense band 2959 cm^{-1} and medium intensity band at 1464 cm^{-1}. The very weak band at 800 cm^{-1} was attributed to the rocking movement of methylenic part. The corresponding C-C vibration was shown as weak intense band at 1062 cm^{-1}. These IR stretching's were good agreed with reported values (Muhit *et al.*, 2010).

The ^{1}H-NMR data of CEC-2 it was seen that H-3 proton appeared at δ 3.52 as a triplet of a double doublet with a J value of 4.5 and 1.1 MHz and H-6 olefinic proton showed a multiplet at δ 5.35. Moreover, six methyl protons appeared at δ 1.26, δ 1.17, δ 1.03, δ 1.00, δ 0.91 and δ 0.90 (3H each, s, CH_3) for H-19, H-18, H-26, H-27, H-21, H-29, respectively. These assignments are in good agreement for the structure of β-sitosterol (Habib *et al.*, 2007; Patra *et al.*, 2010; Muhit *et al.*, 2010; Trivedi and Choudhrey, 2011).

CONCLUSION

From ethanol extracts of leaves of *Corchorus fascicularis* L. using column chromatography resulted two new steroids stigmasterol (CEC-1) and β-sitosterol (CEC-2. As per our aim these compounds were characterized by using physical, chemical and modern spectral analysis. These compounds are shows various pharmacological activities. In future, the active medicaments can be studied further for their pharmacological and biological activities.

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REFERENCES

Bahl, B.S. and A. Bahl, 1992. A Text Book of Organic Chemistry. 13th Edn., Schand and Company Ltd., New Delhi, pp: 11-14.

Habib, M.R., F. Nikkon, M. Rahman, M.E. Haque and M.R. Karim, 2007. Isolation of stigmasterol and beta sitosterol form methanolic extract of root of bark of *Calotropis gigantean* (Linn.). Pak. J. Biol. Sci., 10: 4174-4176.

Harborne, J.B., 1998. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd Edn., Chapman and Hall, London, ISBN-13: 9780412572708, pp: 129-138, 302.

Hartati, S., L.B.S. Kadono, S. Kosela and L.J. Harrison, 2008. A new pyrano xanthone from the stem barks of *Garcinia tetrandra* Pierre. J. Boil. Sci., 8: 137-142.

Hossen, M., M.S. Ali, M. Begum, A. Khatton and A. Halim, 2008. Study on high yield of quality jute seed production for diversified uses. J. Innov. Dev. Strategy, 3: 71-73.

Ian, R., L.D. John, A.D.H. Clague and L.J. Mulheirn, 1976. The 220 MHZ. NMR spectra of phytosterols. Phytochemistry, 15: 195-200.

Jain, P.S., S.B. Bari and S.J. Surana, 2009. Isolation of stigmasterol and γ-sitosterol from petroleum ether extract of woody stem of *Abelmoschus manihot*. Asian J. Biol. Sci., 2: 112-117.

Jain, P.S. and S.B. Bari, 2010. Isolation of lupeol, stigmasterol and campesterol from petroleum ether extract of woody stem of *Wrightia tinctoria*. Asian J. Plant Sci., 9: 163-167.

Kirtikar, K.R. and B.D. Basu, 1996. Indian Medicinal Plants. International Book Distributor, Dehradun, India, pp: 401-402.

Li, C., P.B. Bu, D.K. Yue and Y.F. Sun, 2006. Chemical constituents from roots of *Ficus hirta*. Zhongguo Zhong. Yao. Za. Zhi., 31: 131-133.

Muhit, M.A., A.M. Tareq, A.S. Apu, D. Basak and M.S. Islam, 2010. Isolation and identification of compounds from the leaf extract of *Dillenia indica* Linn. Bangladesh Pharm. J., 13: 49-53.

Nadkarni, A.K., 2005. The Indian Materia Medica. Popular Prakashan Publishing, Mumbai, India, Pages: 378.

Patil, D.A., 2003. Flora of Dhule and Nandurbar Districts. Sing Bishen Publishers, Dehradun, India, pp: 112-113.

Patra, A., S. Jha, P.N. Murthy, Manik and A. Sharone, 2010. Isolation and characterization of stigmast-5-en-3β-ol (β-sitosterol) from the leaves of *Hygrophila spinosa* T. Anders. Int. J. Pharm. Sci. Res., 1: 95-100.

Rajput, A.P. and T.A. Rajput, 2011a. Antibacterial and antifungal activity from leaves extract of *Corchorus fascicularis* Lam. Int. J. Pharm. Tech. Res., 3: 2195-2198.

Rajput, A.P. and T.A. Rajput, 2011b. Pharmacognostic and preliminary phytochemical of Investigation *Corchorus fascicularisl* L. leaves. Int. J. Pharm. Pharm. Sci., 3: 365-366.

Sing, M. and H. Panda, 2005. Medicinal Herbs with their Formulations. Daya Publication House, New Delhi, India, pp: 289- 290.

Singh, V.K., J.N. Govil, S. Hashmi and G. Singh, 2003. Recent Progress in Medicinal Plants, Vol.7: Ethnomedicine and Pharmacognosy II. Stadium Press, USA., pp: 353-355.

Stahl, E., 1969. Thin Layer Chromatography: A Laboratory Handbook. 2nd Edn., Springer, New York.

Tariq, M., S.L. Bhardwaj, R.C. Sharma, S.K. Gupta and L. Gupta, 1973. Pharmacological studies of the glycoside isolated from *Corchorus fascicularis* Lam. Indian J. Exp. Biol., 11: 248-249.

Trivedi, P.C. and N. Choudhrey, 2011. Isolation and characterization of bioactive compound β-sitosterol from *Withania Somnifera* L. J. Pharmacy Res., 4: 4252-4253.