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Isolation and characterization of phytoconstituents from chloroform extract of *Soymida febrifuga* A Juss leaves

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Abstract
Preliminary phytochemical analysis of the *Soymida febrifuga* plant revels that, it contains number of secondary metabolites as an active ingredients useful in various ailments. The present work is for the isolation of such constituents from leaves of *Soymida febrifuga*. The chloroform leaves extract of *Soymida febrifuga* used for the isolation and characterization of the phytochemicals. It is carried out by using TLC, column chromatography, GC-MS, ^{1}H-NMR, ^{13}C-NMR etc. Investigation results the isolation and identification of 'Stigmasterol' from chloroform leaves extract.

Keywords: Medicinal plant, *Soymida febrifuga*, therapeutic, solvent, chloroform, characterization, leaves etc.

Introduction
In past the medicinal plant species has been widely used in traditional medicines and today also being considered as a source of development of therapeutic agents for various ailments. It is due to the presence of bioactive constituents like alkaloids, flavonoids, phenolic acids and polyphenols etc. which are considered to provide anti-aging properties and reducing age related issues [1].
Consequently, it is also important to critically evaluate and understand the available scientific information about the traditional medicinal plant species and their importance.
Plant parts like root, stem, leaves, flowers, fruits and seeds contain various types of constituents which are therapeutic in nature. The chemical tests are required for the qualitative chemical evaluation of the crude drug. Such phytochemical tests are for the identification of the powdered drug [2].
Hence it is important and necessary to carry out the scientific study of structural, functional, chemical and physical with biological characteristics of crude drugs with the help of modern physical and chemical techniques.

Materials and Methods
The leaves of *Soymida febrifuga* A Juss were collected and shade dried, prepared a fine powder for extraction processed with scientific protocols [3, 4, 5, 6]. With the help soxhlet apparatus dried crude leaf powder of *s.f* was extracted by using chloroform. The compounds present in chloroform extract were separated and isolated by techniques such as (TLC) thin layer chromatography and column chromatography. Characterization and structure elucidation of compounds were done by using UV spectral analysis, GC-MS and ^{1}H NMR and ^{13}C NMR data [7, 8].

Extraction
The conventional Soxhlet system was used for the extraction of phytoconstituents. About 20 gm of leaves powder was added into 250 ml flask containing chloroform solvent with reflux condenser. After the successful extraction, extract was concentrated on a rotary evaporator.

Separation
TLC profile was developed for the presence of tannins and triterpenes with the help of using several solvent systems like chloroform: methanol (9: 1), chloroform: methanol (7: 3) and

benzene: methanol (9: 1) proportion. Spots of TLC were visualized by sparing reagent such as $FeCl_3$ Solution and alcoholic H_2SO_4 solution (Sanjay *et al.*, 2013). Colum chromatography was performed on chloroform extract by using Toluene: Chloroform: Ethanol (4:4:1) and collected 85 samples of fractions [9].

Characterization

The characterization of isolated fractions and compounds of different extract of leaves of Soymida febrifuga were done and there structure were identified by physical, chemical, spectroscopic technique like UV, FTIR, GC-MS and ^{1}H-NMR and ^{13}C-NMR analysis [10]. TLC of Fractions collected from Column Chromatography of chloroform extract was named as Compound BCK/CHL/01 and used for further characterization procedures.

Result and Discussion

Soymida febrifuga A Juss. (Meliaceae) an indigenous medicinal plant commonly found in all over the India [11, 12, 8].

Characterization of BCK/CHL/01

White Crystal, with Melting Point 165-167 °C, yields 69 mgs with positive test of Salkowski and Liebermann-Burchard Test for tannins and triterpenes. UV spectra shows characteristic absorption band (λmax) at 256 nm. GC MS spectral data base peak m/z [M+1] at 413.4, which deduced the molecular formula $C_{29}H_{48}O$. Ion peaks in compound is also observed at 144, 198, 229, 243, 264, 265, 289, 346, 382, 391 and 396.

FTIR Data

In the IR spectrum of compound BCK/CHL/01, a very intensely broad band at 3335 cm^{-1} and moderately intense band at 1193 cm^{-1} 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 were observed at 879 cm^{-1}. The corresponding C=C vibrations were 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 bands at 777 cm^{-1} were attributed to the rocking movement of methylene part. The corresponding C-C vibrations were shown as weak intense band at 1036 cm^{-1}.

IR (ranges in cm-1): 3335 (O-H str.), 2934 (C-H str.), 2866 (C-H str.), 1667 (C=C str.), 1459 (C-H bend.), 1193 (O-H bend.), 1089 (C-C Str.), 1036(C-Cstr), 777(CH2 rocking), 667(O-H bend.)

The ^{1}H-NMR Data

In ^{1}H-NMR spectrum of compound BCK/CHL/01 showed H-3 proton appeared as a triplet of a double doublet at δ 3.51 and H-6 olefinic proton showed a multiple 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 32, 33. 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, -CH3).

^{1}H NMR (400 Hz, CDCl3) δ

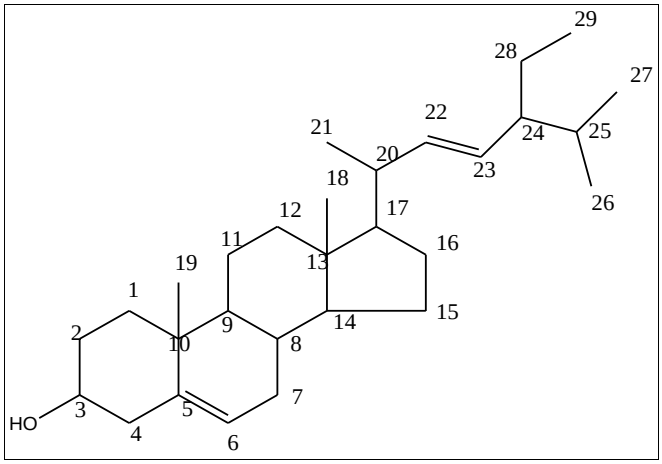
7.30 (s, OH-3), 5.14 (m, 1H, H-6), 4.60 (s, 1H, H-22), 4.23 (s,1H, H-23), 3.51 (tdd, H-3, H-3), 1.21 (s, 3H, H-19), 1.17 (s,3H, H- 28),1.03(s, 3H, H-27), 0.99 (s,3H, H-26), 0.97 (s, 3H, H-24), 0.90 (s, 3H, H-29).

The ^{13}C-NMR- Data

The ^{13}C-NMR spectrum of compound BCK/CHL/01 disclosed the presence of 29 carbon signals. It showed six signals for -CH3 groups in the range of 12.2-21.3 δ. It showed nine signals for - CH2- in the range of 21.5- 39.9 δ. It showed seven signals for –CH- groups in the range of 40.7-51.4 δ. It showed two signals for quaternary carbons at 51.4 δ. It showed one signal for carbon attached to –OH group at 71.9 δ. It showed four signals for two >C=C< at 121.89, 129.50, 138.53, 140.9 δ.

^{13}C-NMR- (CDCl3, 400 MHz) δ

140.9 (C-20), 138.53 (C-21), 129.50 (C-5), 121.89 (C-6), 71.9 (C-3), 57.1 (C-4), 56.2 (C-17), 51.4(C-10, C-13), 50.40 (C-9), 42.5 (C-25), 42.4 (C-22), 40.7 (C-14), 39.9 (C-18), 37.5 (C-1), 32.1 (C-7), 31.9 (C-8), 29.1 (C-2), 25.6 (C-15, C-1 27), 12.4 (C-28), 12.3.



Structure of Stigmasterol

Conclusion

With the help of spectroscopic and phytochemicals assignments it is considered that the structure for the compound BCK/CHL/01 is the structure of **"**Stigmasterol" in *Soymida febrifuga* leaves extract.

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