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Isolation and Characterization of Steroidal Glycoside from Chloroform Extract of the Stem Bark of *Moringa Pterygosperma* Gaertn

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ABSTRACT*:* General phytochemical screening of the chloroform extract of the stem bark of *Moringa pterygosperma* (Moringaceae) revealed the presence of steroids, alkaloids, terpenoids, phenolic compounds, fatty acids, saponins. The aim of the study is to identify and characterize the bioactive constituents from the stem bark of the plant. It has wide folk medicinal use. Isolation of the compound was carried out by chromatographic techniques; isolated compound was purified by chloroform. The isolation and purification afforded white crystalline powder which was subjected to physical, chemical and different spectral analytical methods. The spectral data of UV-Vis, FT-IR, ^{1}H-NMR, ^{13} C NMR and GC-MS of the isolated compound is in support of presence of steroid Nucleus. The compound has been characterized as β-Sitosterol 3-O-β -D- galactopyranoside.

KEYWORDS*:* *Moringa pterygosperma*, phytochemical screening chloroform extract, spectral analysis, steroidal glycoside.

I. INTRODUCTION

Medicinal plants represent a rich source of drug leads. In India and other countries of the world, phytomedicines have been use since time immemorial to treat various ailments long before the introduction of modern used in many parts of the world especially in areas where people do not have access to modern medicines **[1-2].** The human race over the countries has developed a wide variety of technologies and has acquired extensive knowledge from nature and the ecosystem. Exploration of medicinal properties of plants through careful observation and usage had created culture and civilizations. Natural substances of plant origin have been used and are being used throughout the world for human and animal healthcare **[3].** It is important to make a good selection of solvent in the study of activities of plant constituents or active ingredients. In recent days, scientific and technological development have made it possible in the investigation of a large number of medicinal plants by employing a systematic screening method using chromatographic techniques and spectroscopic to establish the actual effort **[4].**

Moringa pteygosperma Gaertn (Moringaceae) is a highly valued plant, distributed in many countries of the tropic and subtropics **[5].** It has an impressive range of medicinal uses with high nutritional value. Different parts of this plant certain a profile of important minerals and are a good source of proteins, Vitamins, B-carotene, amino acid and various phenolics compound. A wide range of chemical constituents including quercetin, kaempferol, alkaloid, vanillin, 4-hydroxymellin and octacosanoic acid and terpenoids are present in this species. Various parts of this plant such as the leaves, roots, seeds, stem bark, fruit, and flowers as cardiac and circulatory stimulants, antitumor, antipyretic, analgesic antiulcer **[6**] antispasmodic, anti inflammatory, diuretic, **[7]** antioxidant **[8]** anti diabetic, antibacterial, antifungal activities **[9-10]** and are being employed for the treatment of different ailments in the indigenous system of medicine. Till date steroidal glycoside from *Moringa pterygosperma* has not been reported hence this study was an attempt to isolate steroids component from the stem bark. Steroids are natural high molecular weight glycosides of triterpene with a very wide distribution in the plant kingdom. Steroids exhibited a range of biological activities. On the other hand steroid also has beneficial pharmaceutical effects.

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The purpose of this study is to identify and characterize the bioactive principles from the stem bark of *Moringa pteygosperma*. In the present investigation we describe the isolation of β- sitosterol-3-O-β-D- galactopyranoside from the chloroform extract of the stem bark of *Moringa.* To the best of our knowledge this compound has not been previously isolated or reported from the stem bark of this variety.

II. MATERIALS AND METHODS

1.Collection of plant material:

Maringa pterygosperma Gaertn plant stem bark where collected from the Devarikhurd, Bilaspur Disttrict of Chhattisgarh, India in the month of October and November 2012. The plant voucher specimens identification was done with the help of professor N.K.Singh, Department of Botany, Govt. Science Post Graduate College, Bilaspur, and C.G. India.

2. Extract and Isolation of compound:

The air-dried stem bark plant- materials (500gm) of *Moringa pterygosperma* were coarsely powdered and extracted in a soxhlet apparatus with chloroform for 12 hours. The chloroform extract was concentrated to obtain a dark viscous mass (63g). A little amount of the extract was sereened chemically for determination of different phytoconstituents. The concentrated extract was dissolve in little quantity of chloroform and adsorbed on silica gel (60-120 mesh) for preparation of slurry. The slurry was then air-dried and chromatographed over a silica gel column. The column was eluated with chloroform-ethyl acetate (8:2, v/v). The chromatograms when developed in iodine chamber. The fractions were collected separately and matched by TLC to check their homogeneity. The fractions were same Rf value were combined together and crystallized. The compound was then recrystallized with methanol and finally purified by preparative TLC. The isolated compound was subjected to various physical and spectral studies for characterization.

3. Phytochemical screening:

Chemical tests for the screening and identification of bioactive chemical constituents like alcohol, carbohydrates, glycosides, steroids, in the medicinal plants under study were carried out in extracts by using standard procedure **[11-12].**

Test for alcohol:

4 g of cerric ammonium Nitrate was dissolved in 10 ml of 2N HNO_3 on mild heating. A few crystals of isolated compound were dissolved in 0.5 ml of dioxane. The solution was added to 0.5ml of cerric ammonium nitrate reagent and dilute to 1 ml with dioxane and shaken well. The developed yellow to red color indicates the presence of an alcoholic hydroxyl group.

Test for Steroid:

1. *Salkowski reaction*: A few crystals were dissolved in $CHCl_3$ and few drops of cemented sulfuric and were added to the solution. A reddish color was seen in the upper $CHCl_3$ layer.

2. *Liebermann- Burchard reaction:* - a few crystals were dissolved in $CHCl_3$ and a few drops of concentrated sulfuric acid were added to it followed by addition of 2-3 drops of acetic anhydride. Solution turned violet-blue and finally green.

Test for glycosides:

1. Raymond's test: A few crystals dissolved in $CHCl_3$ and treated with dinitrobenzene i hot alkali, pink to violet color will be observed indicating the presence of glycosides.

2. Legal's test: A few crystals dissolved in $CHCl_3$ and treated with pyridine and alkaline sodium nitro prusside was added, appearance of cherry red color indicating the presence of glycosides.

Test for carbohydrates:

A few crystals were dissolved in 5ml of distilled water and added with few drops of Molisch's reagent, followed by addition of 1ml of concentration H_2SO_4 by the side of the test tube. After two minutes, 5ml of distilled water was added. Red or dull violet color formation at the interphase of the two layers was taken as positive test.

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Characterization of the compound (1):

White crystalline powder (100 mg) compound; homogenous on TLC, R_f. 0.42, 0.30, 0.20; Found C= 72.91%, H = 10.41%; calculated for $C_{35}H_{60}O_6$ C= 72.97% H= 10.60%; mp. 263-265^{0}C; ***IR (KBr):*** 3373, 2941, 2868, 1642, 1457, 1381, 1038, 881 cm-1; ***^{1}H NMR*** ($CDCl_3$-300MHz): 1.85(2H, m, H-1), 1.80(2H, m, H-2), 3.50(1H, m, H-3), 2.29(2H, m, H-4), 5.35(1H, t, J=6.90Hz, H-6), 1.96(2H, m, H-7), 1.67(1H, m, H-8), 1.10(1H, m, H-9), 0.97(2H, m, H-11), 1.74(2H, m, H-12), 1.08(1H, m, H-14), 1.98(2H, m, H-15), 1.46(2H, m, H-16), 0.90(1H, m, H-17), 0.96(3H, s, H-18), 1.00(3H, s, H-19), 1.35(1H, m, H-20), 0.92(3H, d, J=6.30Hz, H-21), 1.36(2H, m, H-22), 1.16(2H, m, H-23), 0.95(1H, m, J= 7.00Hz, H-24), 1.67(1H, m, J=7.8Hz, H-25), 0.81(3H, d, H-26), 0.84(3H, d, J= 7.00Hz,H-27), 1.27(2H, m, H-28), 0.84(3H, t, J= 8.00,9.00Hz, H-29), 4.29(1H, d, Gal-1), 3.12(1H,dd, Gal-2), 3.32(1H, dd, J= 1.00, 9.00Hz, Gal-), 3.32(1H, dd, J= 1.00, 7.00Hz, Gal-4), 3.18(1H, m, Gal-5), 3.63(1H, dd, J= 3.00, 12.00Hz, Gal-6); ***^{13}C NMR*** ($CDCl_3$-300MHz): 37.4 (C-1), 29.8 (C-2), 79.63 (C-3), 39.00(C-4), 140.2 (C-5), 122.30 (C-6), 32.05(C-7), 32.00(C-8), 50.20(C-9), 36.60(C-10), 21.18(C-11), 39.8(C-12), 42.8(C-13), 56.60(C-14), 24.40(C-15), 28.38(C-16), 56.13(C-17), 12.20(C-18), 18.90(C-19), 36.20(C-20), 18.90(C-21), 34.00(C-22), 26.16(C-23), 45.98(C-24), 29.24(C-25), 19.12(C-26), 18.99(C-27), 23.16(C-28), 12.09(C-29), 101.10(Gal-C-1'), 73.40 (Gal-C-2'), 76.30 (Gal-C-3'), 70.20(Gal-C-4'), 75.6(Gal-C-5'), 61.70(Gal-C-6').

Acid hydrolysis of steroidal glycoside (I):

To the glycoside of I (50 mg) dissolved in minimum amount of ethanol (20ml), sulfuric acid 7% (15ml) was added and refluxed an a water both for four hrs. The ethanol was removed by distilling the reaction mixture under reduced pressure and the reaction mixture was diluted with water (10ml) and extracted with chloroform extract. The chloroform extract was washed with water, dried over anhydride sodium sulphate and concentrated ten times. TLC examination of the residue showed several spots and the major product was purified by preparative TLC using chloroform –ethyl acetate (8:2) as developing solvent. It was crystallized from ethanol as white needles (10mg), mf. $C_{29}H_{50}O$, mp.138-139^0 [414,M+]. It gave positive Solkowski and Liebermann burchard test. It was identified as β-sitosterol on the basis of IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy and other physical properties.

III. RESULT AND DISCUSSION

From the positive for steroids and alcohols given by compound (I), it is assumed to be a compound containing steroidal nucleus. The compound (I) is white crystalline needles like substance with melting point 263-265^{0}C. Its IR spectrum showed on absorption peak in the region 3373 cm^{-1} indicating the presence of a hydroxyl group (OH) and the absorption bands at 2900-2850 cm^{-1}, indicated the presence of –CH aliphatic asymmetric stretching of $–CH_3$, $-CH_2$- and $>CH_2$ groups. The absorption frequencies at 1038 cm^{-1} signifies cycloalkane. The out of plane C-H vibration of unsaturated part was observed at 881 cm^{-1}.The ^{1}H-NMR spectrum showed the chemical shift at 50.96 and 1.00 indicated the presence of two angular methyl signals. The proton NMR spectrum also exhibited one olefinic double bond proton as a double at 5.35, along with the two up field signals of 0.96 and 1.00 respectively, due to the presence of two secondary methyl groups at position 26 and 27 of the skeleton. The very up field chemical shift at 50.85 as a triplet with the intensity of 3H and coupling constant If J = 8.0 Hz was assigned for the terminal methyl group of twenty nine [**13-14**]. Similarly, the other up field chemical shift at 50.92 with the coupling content J=6.30Hz of 3H intensity was assigned the secondary methyl group at position C-21 of the molecular structure. The chemical shift s in the region s 3.18-4.29 as a multiplet was assigned the presence of six protons of the sugar moiety and the very down field chemical shift at s 7.25 was assigned for the proton of OH group of glycoside.

The ^{13}C NMR spectra of the compound (I) revealed the presence of twenty nine carbons, the chemical shift at 19.12 and 18.99 were assigned for the two separate terminal methyl group ($-CH_3$) linked at position C-25 of the molecular structure. ^{13}C NMR has shown recognizable signals 140.2 and 122.30 ppm, which are assigned C-5 and C-6 double bond respectively as in spirostene. The value at 18.9 ppm corresponds to angular carbon atom C-19. Spectra show twenty nine carbon signals including six methyl's, nine ethylene's, eleven methane and three quaternary carbons. The very down field chemical shift at 101.1, 73.4, 76.3, 70.2, 75.6 and 61.7 were assigned for the carbon of the sugar moiety. The C-3 signal of the steroidal glycoside moiety when compared with aglycone had undergone significant downfield shift of 79.63ppm and a up field shift on C-2 and C-4 of 29.8 and 39.00ppm respectively with a more appreciable extend on C-2 and other relative carbon atom provided the conclusive proof for the glycosilation on at C-3.

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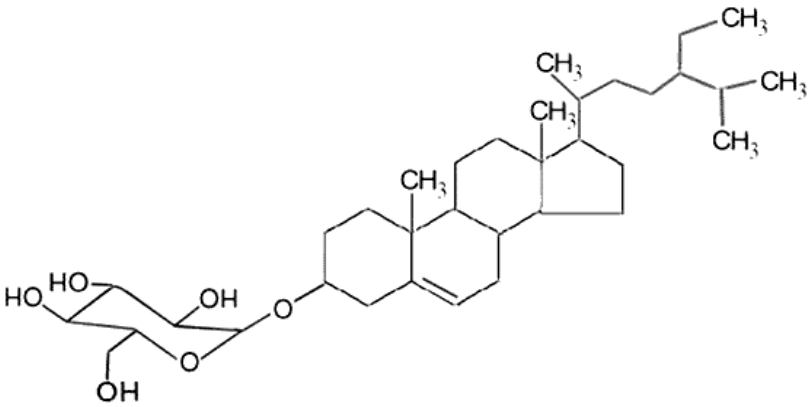
On the basis of IR, ^{1}H NMR, ^{13}C NMR, spectral data and the other physical properties the isolation pure compound (I) were identified and established as β - sitosterol- 3-O-β-D galactopyranoside as shown in fig.1.



Compound (I): β - sitosterol- 3-O-β-D- galactopyranoside

IV. CONCLUSION

From the above finding β- sitosterol- 3-O-β-D- galactopyranoside was isolated from chloroform extract of the stem bark of *Moringa pterygosperma* and chemical structure elucidated respectively .It was carried out by means of various physical(solvent extraction, TLC, column chromatography) and spectral techniques.

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