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An Amazing Traditional Healer of Manipur *Solanum Xanthocarpum* (Leipungkhanga)

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ABSTRACT
Solanum xanthocarpum (Solananceae) is traditionally used as pungent, bitter, digestive, diuretic, alternative, astringent and anthelmintic . It was also used in fever, cough, flatulence and heart diseases. Phytochemistry screening of the aqueous extract of fruits of Solanum Xanthocarpum revealed the presence of steroids, terkenes, phenolic compound, saponins, fatty acids, alkaloids. Isolation of two white crystalline powder which were subjected to physical, chemical and spectral identification using IR,^{1}H-NMR,^{13}C-NMR.
Keywords: *Solanum xanthocarpum, Salasodine, Phytochemical, β-sitosterol, Stigmasterol & Pharmacotherapeutics.*

INTRODUCTION

Solanum xanthocarpum (Leipungkhanga) belonging to family Solanaceae is a herb which grows as a wild plant in many parts of India particulary in hills and valley of Manipur . Different parts of this plant have been used traditionally for curing various ailments such as fever, cough, asthma and diabetes in Indian traditional medicines [1]. The hot aqueous extract of dried fruits is used for treating cough, fever and heart diseases [2]. The plant is extensively studied for the various pharmacological activities like antiasthmatic [3], hepatoprotective [4], cardiovascular [5], hypoglycemic [6], antiulcer [7] and other properties. Scientific evidence in favor of the traditional use of the fruits of *Solanum xanthocarpum* for the treatment of diabetes mellitus has been reported 8. The total extracts and steroidal saponins of *Solanum xanthocarpum*, have been reported to exhibit potent antistress-adaptogenic effects [9].

To prove traditional claim of this plant preliminary immune modulatory evaluation of aqueous extract of fruits of *Solanum xanthocarpum* on cyclophosphamide induced immune suppression was carried out and the results indicated good protection by showing increase in all the hematological parameters [10]. In continuation of the work, it was proposed to isolate active immune modulating agents from the extract. The fruits are reported to contain several steroidal alkaloids like solanacarpine [11], Solana-carpidine, solancarpine, solasodine, solasonine [12] and solamargine [13]. Other constituents like caffeic acid [14] coumarins like aesculetin and aesculin [15], steroids carpesterol, diosgenin, campesterol, daucosterol and triterpenes like cycloartanol and cycloartenol are also reported from the fruits [16].

The isolation and characterization of bioactive principles namely β-sitosterol and stigmasterol from aqueous extracts of fruits of *Solanum xanthocarpum*.

MATERIAL AND METHODS

Plant material:-Fruits of the plant, *Solanum xanthocarpum* were collected from the valley of Manipur in May-June. The sample was shade dried and powdered.

Extraction: Powdered plant material was defatted with petroleum ether (60-80°C) in a soxhelet extractor. The marc was dried and refluxed with water for 8 hrs. The aqueous extract was filtered and concentrated using rotary vacuum evaporator

and the dried extract was stored in an air tight container.

Chromatographic Separation: **TLC**: The aqueous extract of fruits was subjected to thin layer chromatography using silica gel as stationary phase and petroleum ether: methanol (1:1) and petroleum ether: chloroform: methanol (5:2:1) as mobile phase. The chromatograms when developed yielded seven and eight spots respectively that showed zones for steroidal nucleus with Liebermann – Buchard visualizing reagent.

Isolation: Column chromatography of fruit aqueous extract (10g) was conducted using silica gel (Mesh 100-200) by wet packing method. The column was run using petroleum ether, ethyl acetate, methanol and water successive by gradient elution technique. TLC was used to monitor the eluates. Similar fractions were pooled together to yield fifteen fractions. Eluates A, and G were worked upon to yield S_5, and S_9 respectively.

S_5 and S_9 yielded single spots when subjected to TLC using several solvent systems including petroleum ether: ethyl acetate (70:30), petroleum ether: methylene chloride (50:20) and Petroleum ether: Chloroform: Methanol (60:30:10) and shows that the compound is homogenous.

S_5 a white amorphous powder (8.3mg) with a melting point (139°C) was subjected to thin layer chromato-graphy using various solvent systems such as petroleum ether: chloroform: methanol (5:3:1), methylene chloride: petroleum ether (50:50) indicated it to be homogenous compound. S_5 was further subjected to IR, ^{1}HNMR and ^{13}CNMR to ascertain the chemical structure.

S_9 a white crystalline powder (9mg) with a melting point (137°C) was subjected to TLC using various solvent systems such as petroleum ether: chloroform: methanol (5:3:1), methylene chloride: petroleum ether (50:50) indicated it to be homogenous compound. S_9 was further subjected to ^{1}HNMR and ^{13}CNMR to ascertain the chemical structure.

Test for proteins and amino acids:-

1. Ninhydrin Test: - Crude extract when boiled with 2ml of 0.2 solution of ninhydrin, violet color appeared suggesting the presence of amino acid and proteins.

2. Xanthoprotein Test: - In 2ml of extract 3 drops of nitric acid were added by the side of the test tube followed by addition of 40% NaOH. Appearance of yellow color indicates the presence of proteins and free amino acids.

The structures were simulated using NMR program to obtain the chemical shifts of both proton and carbon. Phytochemical analysis (Salkowski's test and Lieberman-Burchard test) of both the compounds confirms its steroidal nature.

Spectroscopic Characterization: Different spect-roscopic methods were used to elucidate the structure of isolated compounds. Among the spectroscopic techniques IR, ^{1}H-NMR and ^{13}C-NMR were carried out. The infra red spectrum was recorded on FTIR (model Shimadzu 8700), ^{1}H-NMR and ^{13}C-NMR spectra were recorded using $CDCl_3$ as solvent on Bruker Advance II 400 NMR spectrometer were recorded at high resolution on a mass spectrometer (model Shimadzu) at Sophisticated Instrumentation centre and the data are given in m/z values.

Characterization of S_5 and S_9: The exact molecular mass for the S_5 and S_9 was found to be 414.7 and 412 respectively. Based on this the proposed molecular formula of the compounds could be tentatively: $C_{29}H_{50}O$ and $C_{29}H_{48}O$. From ^{13}CNMR and ^{1}HNMR the number of C and H were found to be near to the formula $C_{29}H_{50}O$ and $C_{29}H_{48}O$. Since, the compounds give positive test for steroids.

Based upon the functional group analysis it was found that the nature of oxygen was hydroxyl, also supported by IR spectroscopy. This implies presence of one double bond in the structure. So, the steroids with other functional groups were rejected. Also on considering the nature of oxygen as hydroxyl and presence of one double bond, the general formulas for the compounds were C_nH_{2n-6}. Therefore, they must be tetra cyclic compounds. Based on the melting point and other related data

(IR, NMR and Mass) the structure of the isolated compounds S_5 and S_9 were proposed as (fig. 1)

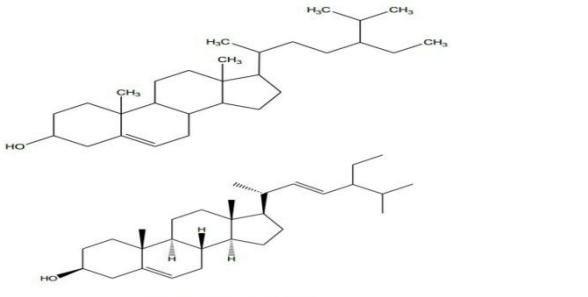

Fig 1. Structure of S_5 and S_9

The compound S_5 is a white amorphous powder, m.p.137°C. IR absorptions bands appeared at 3549.99 cm-1(OH), 2935.73 cm-1 (CH2), 2867.38 cm-1 (CH), 1637.63cm-1 (C=C), 1063.34cm-1 (C-O). Mass spectra of this compound suggested that its molecular mass is 414(M.F. C29H50O) having characteristic fragments observed at m/z: 414, 396, 381, 329, 303, 289, 273,255, 231, 213, 199, 173, 159, 119, 95, 81 and 69. NMR spectrum of this compound resembled data published in previous studies [16-17].

This compound is having six methyl, eleven methylene and three quaternary carbons with a hydroxyl group. The carbons of alkenes conjugated are at 140.78 ppm (C5) and 121.72 ppm (C6) which was confirmed from the ^{13}CNMR. The above IR, ^{1}HNMR, ^{13}CNMR and a comparison of the ^{13}CNMR signal with those described in the literatures 18-20 showed the structure of S_5 to be the β-Sitosterol.

The compound S_9 is a white crystalline substance with a melting point of 139°C. IR absorptions bands appeared at 3384cm-1 (OH), 3218 cm-1 (cyclic olefinic –HC= CH- str), 3025 (=CH str) and 2868 cm-1 assigned to C-H str. Other absorption frequencies include 1665 cm-1 as a result of C=C absorption, however, this band is weak. 1462 cm-1 is a bending frequency for cyclic (CH2)n and 1382 cm-1 for –CH2 (CH3)2γ. The absorption frequency at 1332 cm-1 can be attributed to OH def. and at 1046 cm-1 signifies cycloalkane. These absorption frequencies resemble the absorption frequencies observed for Stigmasterol [20].

The Proton NMR has revealed the existence of signals for olefinic proton at 5.358 (br.,s.), Angular methyl proton at 0.68 (s), 0.699 (s) and 1.01 (s) corresponding to C18 and C19 proton respectively. The ^{13}CNMR has shown recognizable signals 140.8 and 121.7ppm, which are assigned C5 and C6 double bonds respectively as in Δ5 spirostene 19. The δ value at 71.0ppm is due to C3 β- hydroxyl group 20. The signals at δ19.4 and 11.9 ppm correspond to angular carbonatom (C18 and C19 respectively). The above IR, ^{1}HNMR, ^{13}CNMR, LCMS spectral data and a comparison of the ^{13}CNMR signal with those described in the literatures 20-23 shows that the structure of S_9 to be the Stigmasterol.

The structures were simulated using NMR program to obtain the chemical shifts of both proton and carbon. On comparison the experimental data matched with the simulated data which supports the proposed structure of this compound.

RESULTS AND DISCUSSION

Solanum xanthocarpum is a very popular herb among the local people of Manipur for its medicinal properties. The study affirmed that aqueous extract of the fruits of *Solanum xanthocarpum* is effective immune modulatory agent. The extract potentiated the non- specific immune response by increasing the haematological parameter and neutrophil adhesion test, which may attributed to different phytoconstituents. Hence, to isolate the phytoconstituent it was subjected to fractionation and characterizations of isolated compounds.

Two compounds S_5 and S_9 showing positive tests for Steroids and alcohols were isolated. The S_5 is white amorphous powder like substance with melting point 137°C. Whereas, the S_9 is white crystalline substance with melting point 139°C on subjection to IR spectroscopic analysis, the observed absorption frequencies resemble the absorption frequencies observed for β- sitosterol and Stigmasterol. The proton NMR showed the proton environment resembles the protons of β-sitosterol and Stigmasterol. The ^{13}C-NMR has

shown recognizable signals of β sitosterol and Stigmasterol.

The weak molecular ions were given at m/z 414 and 412.7. The molecular weight and fragmentation pattern indicate that the isolated compounds are β-sitosterol and stigmasterol.

The above I.R., ^{1}HNMR, ^{13}C-NMR and LCMS spectral data and their comparison with those described in the literatures showed the structure of isolated compounds are to be the β-sitosterol and stigmasterol [24].

CONCLUSION

This study therefore concludes that the ethanolic extract of the fruits of *Solanuim Xanthocarpum* contains β- sitosterol and Stigmasterol .these compound may be responsible for immune modulatory activity. The qualitative photochemical screening test shows that *Solanum Xanthocarpum* contains proteins and amino acids also.

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