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Chemical Investigation of Flower's of *Nymphaea tetragona*

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ABSTRACT

The flower's of *Nymphaea tetragona* were soxhleted with petroleum ether (b.p. 60-80^0), chloroform and alcohol (95%). We isolated nine higher fatty acids from this herb. Identification of the isolated compounds were carried out through IR, PMR and MASS spectroscopy.

Key words: *Nymphaea tetragona*, IR, PMR, MASS, fatty acids.

INTRODUCTION

Nymphaea tetragona (Nymphaeaceae) is a dwarf aquatic herb found in the Himalayas and also in swamps on khasi hills. The calcium oxalate crystals were reported[1] in the leaves of *Nymphaea tetragona*[2]. we now report the isolation of some higher fatty acids, β-sitosterol, Betulin, Betulinic acid, Kaempferol, Quercetin, Caffiec acid, Kaempferol-3-D-glucoside, Quercetin-3-D-glucoside and β-sitosterol-3-D-glucoside.

EXPERIMENTAL

The flower's were soxhleted successively with petroleum ether (b.p. 60-80^0), chloroform and alcohol (95%). The petroleum ether extract was concentrated under reduced pressure and chromatographed over silica gel. Elution were carried out with petroleum ether and its mixture with benzene furnished the compounds A, B, C, D and fraction X_1 were obtained. The chloroform extract on concentration under reduced pressure and chromatographed over silica gel using petroleum ether and solvents of increasing polarity as eluents. Fraction X_2 and compound G were obtained from benzene and mixture of ethyl acetate-methanol (1:1) eluates respectively. Fraction X_1 (From petroleum ether extract) and Fraction X_2 showed similar TLC behaviour, were mixed together and resolved by preparative TLC using petroleum ether-methylene chloride (1:1) and benzene-chloroform as solvent mixture to two compounds E (lower part) and F (upper part). The eluates with ethyl acetate-methanol was crystallized from pyridine : methanol mixture as compound G. The alcoholic extract on concentration under reduced pressure and divided into ethyl acetate soluble and insoluble fractions. The ethyl acetate soluble part when chromatographed over silica gel, furnished the compound H, I and J. The ethyl acetate insoluble fraction was extracted with methanol and after concentration under reduced pressure, was separated by preparative TLC (EtOAc:MeOH: H_2O,25:4:3, v/v) into compound K (high Rf) and L (low Rf).

RESULTS AND DISCUSSION

Compound A

Elution with petroleum ether-benzene (4:1) yielded yellowish oily liquid, solidifying point 13° and boiling point 285°C and 100 mm. its alcoholic solution showed acid reaction to litmus; decolourized bromine and $KMnO_4$ solution. The IR band at 3100-2600 (br), 1705,1650,1410,1292,1245,1186 & 938 showed that it has an unsaturated fatty acid. It was identified as oleic acid and further confirmed by

preparation of the dibromide, solidifying point 28^0 C[3] and Amide, m.p. 76-78^0 C[4].

Compound B

Petroleum ether-benzene (3:2) elution yielded colourless plates (MeOH:Me_2CO), m.p. 138^{0}C; positive Liebermann-Burchard and TNM test; the acetate, m.p. 123-124^{0}C. Identification of the compound as -sitosterol was made through comparison of m.p., Co-TLC, IR and PMR spectra.

Compound C

Further elution with benzene: ethyl acetate (3:1) gave white needles ($CHCl_3$:MeOH), m.p. 250^{0}C, positive Liebermann-Burchard and TNM test; the acetate, m.p. 217-218^{0}C; $_{max}$ (KBr) 3490-3380 (br), 3220, 2920, 2860, 1640, 1480, 1460, 1100, 1025, 870, 770 and 620 cm^{-1}. The PMR[5] and Mass[6] spectral data identified it as betulin which was further confirmed by m.p., Co-TLC with authentic sample.

Compound D

The benzene-ethyl acetate (1:2) elution yielded white solid crystals (Bz:EtOAc) m.p. 314-315^{0}C; positive Liebermann-Burchard and TNM test; decolourized bromine in chloroform; the methyl ether, m.p. 223-225^{0}C. Identification of the compound as betulinic acid was made through comparison of spectral (IR[7], PMR, MASS[8]) analysis.

Compound E

From lower part of preparative TLC, white solid was obtained which was recrystallised from alcohol as white crystals, m.p. 56-57^0 C, no colouration with TNM and $FeCl_3$ solution. It was identified as myrsitic acid Co-TLC and Co-IR with an authentic sample. Further confirmed by preparation of its amide, m.p. 101-102^{0}C, anilide, m.p. 84-85^{0}C[9].

Compound F

From upper part of preparative TLC, white solid was obtained which was crystallised from ethanol as colourless crystals, m.p. 68-69^0 C, no colour with TNM and $FeCl_3$ solution. It was identified as stearic acid by chemicals and spectral analysis and Co-TLC with an authentic sample. Further confirmed by preparation of its amide, m.p. 108-109^{0}C, anilide, m.p. 92-93^{0}C.

Compound G

The eluates with ethyl acetate-methanol was crystallized from pyridine : methanol mixture as colourless plates, m.p. 288-290^0 C; positive Liebermann-Burchard and TNM test; positive Molisch test and on acid hydrolysis, it gives -sitosterol-3- glucoside by m.p., Co-TLC, chemical and spectral analysis. Further confirmed by preparation of its tetraacetate, m.p. 169-170^{0}C.

Compound H

Elution with benzene-ethyl acetate (3:2) afforded yellow needles (hot aqueous ethanol), m.p. 276-277^{0}C; positive shinoda and pew test; brownish green colour with alcoholic $FeCl_3$, yellow under UV and UV/NH_3;λ_{max} (MeOH) 250sh, 266, 365 nm. Its IR data revealed it to be Kaempferol which was further confirmed by preparation of methyl ester, m.p. 150-151^{0}C.

Compound I

The Elute with benzene-ethyl acetate (1:1) was crystallized as yellow needles (ethanol), m.p. 315-316^{0}C; Rf=0.84 (solvent system n-BuOH:AcOH:H_2O,4:1:5,v/v); positive shinoda and pew test; olive green colour with alcoholic $FeCl_3$; yellow under UV and UV/NH_3;λ_{max} (MeOH) 255sh, 268, 302sh, 370 nm. Its IR and mass data revealed it to be quercetin. It was further confirmed by m.p., Co-PC and Co-IR with an authentic sample and preparation of its penta acetate, m.p. 198-199^{0}C.

Compound J

Elution with ethyl acetate-methanol (1:1) afforded yellow needles, m.p. 195-196^{0}C; green colour with $FeCl_3$; decolourised bromine and $KMnO_4$ solution; green precipitate with baryta solution[10];λ_{max} (MeOH) 215, 288, 315 nm. Its IR data revealed[11] it to be caffeic acid which was further confirmed by the preparation of the methyl ester, m.p. 150-151^{0}C and diacetate, m.p. 191-192^{0}C.

Compound K

The compound as yellow solid, was recrystallized from ethanol or pale yellow crystals, m.p. 176-178^{0}C; Rf=0.85 (solvent system n-BuOH:AcOH:H_2O,4:1:5,v/v); positive shinoda and pew test; brownish red colour with alcoholic $FeCl_3$; positive Molisch test. On acid hydrolysis yielded Kaempferol and glucose;λ_{max} (MeOH) 265sh, 355

nm. It was identified as Kaempferol-3-glucoside. It was further confirmed by hydrolysis with emulsion.

Compound L

The compound L was recrystallized from methanol as yellow needles, m.p. 234-236^{0}C; Rf=0.79 (solvent system n-BuOH: AcOH: H_2O,4:1:5,v/v); positive shinoda and pew test; brownish colour with alcoholic $FeCl_3$; positive Molisch test; dull brown under UV and yellow in UV/NH_3;λ_{max} (MeOH) 255, 370 nm. Its IR data revealed it to be quercetin-3-glucoside which was further confirmed by the preparation of the acetate, m.p. 134-135^{0}C and acid hydrolysis as well as hydrolysis with emulsion yielded quercetin and glucose in equimolar ratio. The identity was further confirmed by m.p., Co-PC and Co-IR with the authentic sample.

CONCLUSIONS

The calcium oxalate crystals were previously reported in the leaves of *Nymphaea tetragona*. we isolated some of the higher fatty acids from the flower's as, β -sistosterol, Betulin, Betulinic acid, Kaempferol, Quercetin, Caffeic acid, Kaempferol-3-D-glucoside, Quercetin-3-D-glucoside and β -sitosterol-3-D-glucoside.

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