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Extraction and isolation of compounds from the flowers of Nelumbo Nucife

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ABSTRACT

The flowers of Nelumbo Nucifera were soxhleted with n-hexane, diethyl ether and alcohol (95%). We isolated higher fatty acids and some other compounds. Identification of the isolated compounds were carried out through chromatography, IR PMR and Mass spectroscopy.

KEYWORDS

Lal kamal;
Padam;
IR;
PMR;
Fatty acids.

INTRODUCTION

The plant Nelumbo Nucifera belongs to the family Nymhaeaceae. The plant is distributed throughout the warmer parts of India. It is a large aquatic herb with slender, elongate branched, creeping stems. Sending out roots at the nodes. Leaves membranous 0.3-0.6 m or more in diameter, orbicular, concave, erect, exactly peltate[1], glabrous, petioles very long. The flower is sweet and cooling, it allays cough, thirst, blood defect, skin eruptions and beneficial to the eyes.

EXPERIMENTAL

The flowers of Nelumbo Nucifera (1.5kg) were exhaustively extracted with n-hexane, diethyl ether and alcohol (95%). The n-hexane extract was concentrated on water bath under reduced pressure and removal of the solvent a crude yellow solid mass (13gm) was obtained. It was dissolved in n-hexane (a part of the residue remain insoluble in cold solvent was rejected) and after concentration, chromatographed on silica gel (450 gm) column. The elutions were carried out with n-hexane and mixture of solvents of increasing polarity. Different fractions f1, f2 and f3 having compounds A, B and C respectively. The diethyl ether extract on concentration under reduced pressure and chromatographed over silica gel using petroleum ether and solvents of increasing polarity as eluents. Fraction f4 having compound D and the compound E were obtained from benzene: ethyl acetate (3:2) and mixture of ethyl acetate: methanol (1:1) eluates respectively. Similarly the flowers were extracted with 95% alcohol. The extract was concentrated and cooled. The dirty white crystals (compound F) were separated by filtration. The filtrate was further concentrated and divided into water soluble and insoluble part. The water insoluble part was extracted with ethyl acetate which furnished yellow compound G. the

water soluble part was not identified (mp 171^{0}C) due to small amount.

RESULT AND DISCUSSION

Elutions were carried out with n-hexane and mixture of solvents of increasing polarity which afforded compounds A, B and C respectively.

Compound A

The compound A, white leaflets, m.p. 59-60^{0}C gave a single spot on TLC and was analysed as the molecular formula C_{18}-$H_{38}O$. It neither gave coloration with TNM and $FeCl_3$ solution nor decolorized bromine and $KMnO_4$ solution. The IR spectrum of the compound is comparable to that of n-dodecyl alcohol[2]. The IR band at 2907, 2841, 1466, 1381, 1263 and 1055 Cm^{-1} confirmed that it was an alcohol. The broad band at 3436 cm^{-1} confirmed the presence of OH group which was further supported by the fact that the compound forms acetate, m.p. 32-33^{0}C and p-nitro benzoate, m.p. 64-65^{0}C whose m.p. agreed with those reported in the literature[3]. The doublet at 730 and 720 cm^{-1} indicated the polymethylene group and thus the compound A was a long chain saturated aliphatic primary alcohol (stearyl alcohol) and finally confirmed by m.mp. and CO-TLC with an authentic sample.

Compound B

Elution with n-hexane:benzene (3:2) yielded colourless liquid, solidifying point 13^0 and boiling point 285^{0}C at 100mm. Its alcoholic solution showed acid reaction to litmus; decolorized bromine and $KMnO_4$ solution. The IR band at 3100, 2600(br), 1705, 1650, 1410, 1292, 1245 and 938 cm^{-1} showed that it has an unsaturated fatty acid. It was identified as oleic acid and further confirmed by preparation of its derivative, amide, m.p. 76-78^{0}C[4].

Compound C

Elution with n-hexane:benzene (1:1) yielded white solid (1gm) which on crystallization with ethanol yielded white crystals, m.p. 61-62^{0}C (lit 62.5^{0}C)[5]. It gave no coloration with TNM & $FeCl_3$ solution. The alcoholic solution of the compound was acidic to blue litmus. The IR spectrum of the compound is similar to that of higher fatty acids[6,7]. The important absorption bands at 1705, 1410, 1292 & 938 cm^{-1} confirm that it is an acid. The broad band at 3100-2600 cm^{-1} & a strong band at 1705 cm^{-1} indicated the presence of COOH group and doublet at 727 and 720 cm^{-1} showed long chain of methylene groups. The absence of band around 1650 cm^{-1} showed that it is a long chain saturated fatty acid. From this data compound C is identified as palmatic acid and finally confirmed by the preparation of its derivatives, amide, m.p. 105-106^{0}C and anilide, m.p. 88-89^{0}C.

The diethyl ether extract gave the compound D & E.

Compound D

Benzene –ethyl acetate (3:2) elution yielded white solid (1.6 gm) which on crystallization with ethanol yielded white crystals m.p. 42-43^{0}C (lit 43^{0}C)[8]. It gave no coloration with TNM & $FeCl_3$ solution. The IR band at 1705, 1410, 1292 and 938 cm^{-1} confirms that it is an acid. The broad band at 3100-2600 cm^{-1} and a strong band at 1705 cm^{-1} indicated the presence of COOH group and doublet at 727 and 720 cm^{-1} showed long chain of methylene groups. The absence of band around 1650 cm^{-1} showed that it is a long chain saturated fatty acid. From the above data the compound D was identified as lauric acid and finally confirmed by CO-TLC and preparation of its derivatives, amide, m.p. 97-98^{0}C and anilide, m.p. 76.5-77.7^{0}C[5].

Compound E

The eluates with ethyl acetate- methanol (1:1) were crystallized from pyridine: methanol mixture as colorless plates, m.p. 288-290^{0}C, positive Liebermann-burchard & TNM test; and positive molisch test and on acid hydrolysis it gives β-sitosterol-3-glucoside by m.p., CO-TLC, chemical and spectral analysis. Further confirmed by preparation of its tetra acetate, m.p. 169-170^{0}C.

The alcoholic extract gave the compound F & G.

Compound F

The white solid, obtained from the removal of the solvent was recrystallized from alcohol as white crystals, m.p. 56-57^{0}C (lit 58^{0}C). It gave no

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coloration with TNM test and $FeCl_3$ solution. The alcoholic solution of the compound was acidic to blue litmus. The IR spectrum of the compound is similar to that of higher fatty acid. From IR data the compound F was identified as myrsitic acid and finally confirmed by CO-TLC and preparation of its derivatives, amide, m.p. 101-102^{0}C and anilide, m.p. 84-85^{0}C.

Compound G

The compound, G obtained as yellow solid, was recrystallized with ethanol as yellow crystals (200 mg) m.p. 177-178^{0}C. It gave deep red color with Mg-HCl and bluish pink color with Zn-HCl and brown red color with alcoholic $FeCl_3$. It also gave positive molisch test for carbohydrates. It was yellow green in UV and dark yellow in UV/ NH_3 and gave yellow color with alkalies. These color reactions indicated the compound to be a flavonal Glycosides. On acid hydrolysis the compound gave aglycone, m.p. 276-278^{0}C (identified as Kaempferal by UV, mmp, CO-IR & CO-PC) and D-glucose (identified by CO-PC) in equimolecular proportion indicating it to be a monoglucoside. From IR and UV data, the compound was identified as kaempferal-3-glucoside and finally confirmed by comparison with authentic sample.

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