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SUMMARY

The brown viscous mass obtained from the concentrated 95% ethanolic extract of the seeds of *Fagopyrum esculentum* was extracted with various solvent and the ethyl acetate soluble part when worked up resulted in the isolation of two new dihydroflavonol glycoside, Aromadendrin-3-O-B-galactopyranoside and Taxifolin-3-O-B-D-xylopyranoside.
TWO NEW DIHYDRO FLAVONOL GLYCOSIDES

FROM FAGOPYRUM ESCULENTUM SEEDS

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Department of Chemistry
UNIVERSITY AT SAGAR
SAGAR (M.P.) 470 003 INDIA

KEY WORD INDEX

Fagopyrum esculentum (Moench), Polygonaceae;
Aromadendrin-3-O-B-galactopyranoside and Taxifolin-3-O-B-D-
Xylopyranoside.

The occurrence of aromadendrin and taxifolin appears to be rare in plants used for food except that of ampelopsin from Ampelopsis meliaeefolia [1], but a recent report about the isolation of dihydro flavonoid glycosides, Astilbin and Angeletin from grapes and wine [2] encouraged us to investigate further the seeds of Fagopyrum esculentum resulting in the isolation of two new dihydro flavonol glycosides.

RESULT AND DISCUSSION

The brown viscous mass obtained from the concentrated 95% ethanolic extract of the seeds of Fagopyrum esculentum [3,4] was extracted successively with different solvents and the ethyl acetate soluble fraction responded to positive tests for flavonoid glycoside [5,6]. It was dried to get an amorphous
mass, which on TLC showed two spots and was, therefore, worked up by column chromatography to get two compounds which were studied separately.

The first compound crystallised from methanol, m.p. 221-222°C, C_{21}H_{22}O_{11}, M^+ = 450, and responded to ferric reaction, Molisch and Shinoda (Mg-HCl) tests and developed an orange-red colour, slowly when zinc was used instead of Magnesium \( \text{H}^+ \) (Pew's modification), indicating that it was a flavanol-3-glycoside.

On hydrolysis with 7% ethanolic H_2SO_4, it yielded an aglycone and D-galactose (by Co-PC and TLC). The aglycone crystallised from methanol m.p. 243-245°C, C_{15}H_{12}O_6, M^+ = 288. Its UV spectrum showed band II at 290 nm, which is characteristic for the dihydroflavonols \( \text{H}^+ \). The dihydro nature was further supported by a yellowish brown colour obtained on treatment with 2,4-dinitro phenyl hydrazine and sodium borohydride with sodium hydroxide solution \( \text{H}^+ \). The bathochromic shift of 58 nm of band I with AlCl_3 is a characteristic feature of hydroxy group at C_3. The bathochromic shift of 42 nm with sodium methoxide indicated the presence of unsubstituted C-5 and C-7 hydroxyl groups. It gave blue colour when NaHCO_3 solution was added to the Shinoda reaction indicating the presence of free hydroxyl group at C-4'. It also gave positive Shinoda test for the presence of free hydroxyl group at C-3, thus confirming its identity as aromadendrin.

The other compound crystallised from ether : ethanol (2:1)
had m.p. 219-221°C, C_{20}H_{20}O_{11}, M^+ = 436, and responded to similar tests as that described for the earlier compound.

On hydrolysis with 7% ethanolic H_2SO_4 it yielded an aglycone and D-xylose (by Co-PC and TLC). The aglycone showed absorptions maxima at λ_{max} 327 nm (I), 285 nm (II), and showed bathochromic shift of 40 nm of band II with NaOMe indicating the presence of hydroxyl groups at C-5 and C-7. The bathochromic shift of 60 nm of band I with AlCl_3 suggested the presence of hydroxyl group at C-3 and hypsochromic shift of 34 nm in band I with AlCl_3/HCl suggested the presence of hydroxyl group at position C_3, C_4. Thus it was identified as Taxifolin.

**EXPERIMENTAL**

The seeds of *Fagopyrum esculentum* (Moench) were supplied by M/s Himalaya Range Drug Fields, Simla and authenticated by the Botany Department of this university, and the vouchers are deposited with the supervisor.

The air dried and powdered seeds were extracted with 95% ethanol and the extract concentrated and resolved into water soluble and insoluble parts. The concentrated water soluble part was extracted with ethyl acetate to get an amorphous mass giving two spots on TLC (Rf 0.52 and 0.63; in chloroform : methanol 3:2).
Compound first had molecular formula C_{21}H_{22}O_{11}, m.p. 221-222°C, M⁺ = 450 and hepta acetyl derivative was prepared with Ac₂O/pyridine molecular formula C_{35}H_{36}O_{19}, m.p. 132-134°C, M⁺ = 744. It showed KBr max 3385, 2900, 1680, 1608, 1450, 1270, 1120, 820 and ¹H NMR (CDCl₃) δ = 3.95 (s, anomeric proton), 4.79 (d, J = 0.6 Hz, H-3), 5.28 (d, J = 10.5 Hz, H-2), 5.90 (s, H-6), 5.91 (s, H-8), 6.78 (2H, d, J = 8.4 Hz, H-3' and H-5'), 7.33 (2H, d, J = 8.5 Hz, H-2' and H-6'), 2.18 (3H, s, OAc-4'), 2.32 (3H, s, OAc-7), 2.45 (3H, s, OAc-5), 2.14 (3H, s, OAc-3''), 2.09 (3H, s, OAc-4''), 2.06 (3H, s, OAc-2''), 1.98 (3H, s, OAc) and 3.5-4.30 (6H, m, protons of sugar residue), and MS M⁺ = 450 and m/e 288, 287, 260, 152, 135 and 124.

The other compound had molecular formula C_{20}H_{20}O_{11}, m.p. 219-221°C, M⁺ = 436 and acetyl derivative prepared molecular formula C_{36}H_{36}O_{19}, m.p. 153-154°C, M⁺ = 772 showed KBr max 3350, 2902, 1682, 1620, 1530, 1452, 1280, 772, and ¹H NMR (CDCl₃) δ = 6.88 (1H, s, H-2'), 6.73 (2H, s, H-5' and H-6'), 5.90 (1H, s, H-8), 5.88 (1H, s, H-6), 5.23 (1H, d, J = 9.9 Hz, H-2), 4.65 (1H, d, J = 9.9 Hz, H-3), 4.32 (1H, s, C₁-anomeric proton), 3.52-4.30, (5H, m, protons of sugar residue), 2.45 (3H, s, OAc-5), 2.38 (3H, s, OAc-3''), 2.36 (3H, s, OAc-7), 2.32 (3H, s, OAc-4''), 2.14 (1H, s, OH-3''), 2.09 (1H, s, OH-4''), 2.04 (1H, s, OH-2'') and MS M⁺ = 436 and m/e 304, 288, 287, 154, 153, 134, 132 and 126.
REFERENCES


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P.T.O.
Thanking you,

Yours sincerely,

Asit K. Datta
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10, D. P. Road, 25 April 1970

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Sagar - 470 003
NEW FLAVANONE GLYCOSIDE; ERIODICTYOL 5-O-METHYL ETHER
-7-O-β-D-GLUCOPYRANOSYL (1→4)-β-D-GALACTOPYRANOside

FROM THE SEEDS OF FAGOPYRUM ESCULENTUM (MOENCH)

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Natural Product Laboratory,
Department of Chemistry
Doctor Harisingh Gour Vishwavidyalaya, Sagar (M.P.), INDIA

Isolation and identification of Eriodictyol 5-O-methyl ether-7-O-β-D-glucopyranosyl (1→4)-β-D-galactopyranoside from the seeds of Fagopyrum esculentum is reported.

Fagopyrum esculentum L. Moench (N.O. Polygonaceae) is found from Kashmir to Sikkim. Its seeds are cooked and eaten as vegetable and grain are used in colic, cholerae, diarrhoea, flaxes and abdominal obstructions. Earlier workers have already reported the occurrence of rutin (8.5%) in Fagopyrum species along with some poisonous substance. The presence of a flavonoidal compound in Fagopyrum esculentum stimulated us to investigate its seeds further, resulting in the isolation of a new flavanone glycoside.

The ethylacetate soluble part when worked up gave positive Molsish test. The compound, molecular formula $C_{28}H_{32}O_{16}$, m.p. 91-93°, and showed spectral shifts $\lambda_{\text{max}}^\text{MeOH}$ 290, 330, $\lambda_{\text{max}}^{(\text{MeOH}+\text{AlCl}_3)}$ 295, 330, $\lambda_{\text{max}}^{(\text{MeOH}+\text{NaOAc})}$ 290, 335, its $\delta_{\text{KBr}}$ 3340, 2910, 1710, 1610, 1540, 1450, 1280, and 750. $^1$H NMR
\[(\text{COCl}_3) \text{ S} = 1.96 \ (3\text{H, S, C}_2\text{, OAc}), \ 2.00 \ (3\text{H, S, C}_3\text{, OAc}), \ 2.02 \ (3\text{H, S, C}_6\text{, OAc}), \ 2.04 \ (3\text{H, S, C}_4\text{, OAc}), \ 2.06 \ (3\text{H, S, C}_6\text{, OAc}), \ 2.08 \ (3\text{H, S, C}_2\text{, OAc}), \ 2.10 \ (3\text{H, S, C}_3\text{, OAc}), \ 2.34 \ (3\text{H, S, C}_4\text{, OAc}), \ 2.42 \ (3\text{H, S, C}_4\text{, OAc}), \ 2.80 \ (2\text{H, q, J=17 Hz, C}_3\text{-2H}), \ 3.76 \ (3\text{H, S, C}_6\text{-OCH}_3), \ 3.82 - 4.62 \ (12\text{H, m, proton of sugar residue}), \ 4.72 \ (1\text{H, d, J=7.0 Hz, C}_1\text{-anomeric proton}), \ 4.94 \ (1\text{H, d, J=7.2 Hz, C}_1\text{-anomeric proton}), \ 5.2 \ (1\text{H, dd, J=5, 11 Hz, C}_2\text{-H}), \ 6.0 \ (1\text{H, d, J=2.5 Hz, C}_6\text{-H}), \ 6.3 \ (1\text{H, d, J=2.5 Hz, C}_8\text{-H}), \ 7.20 \ (1\text{H, S, C}_5\text{-H}, 7.25 \ (2\text{H, d, J= Ha, C}_2\text{-H and C}_6\text{-H}) \text{ and M}^+ \ 626 \text{ and m/e } 463, 447, 302, 274, 259, 167, 166, 138, 136 \text{ and 134.}

On acid hydrolysis with 7% \text{EtOH-H}_2\text{SO}_4 \text{ it yielded an aglycone, galactose and glucose (CO-PC and TLC). The aglycone crystallized from } \text{EtOH as yellow needles, analysed for C}_{16}\text{H}_{12}\text{O}_6, \text{ m.p. 110-111°C, M}^+ = 302. (Found C = 63.42%; H = 4.66%, calculated C = 63.57%; H = 4.63%). The } \lambda_{\text{max}} \text{MeOH 290, 330, MeOH + AlCl}_3 \ 288, 335, \text{ and } \lambda_{\text{max}} \text{MeOH + NaOAc 325, 335 nm.}

The UV spectrum of the aglycone confirmed the presence of a free OH at C-7 (bathochromic shift with NaOAc) and an OMe at C-5.5 \((\text{KBr max}) 3330, 2900, 1712, 1608, 1536, 1452, 1278 \text{ and 750. } ^{1}\text{H}NMR \ (\text{CDCl}_3) \ S = 2.34 \ (3\text{H, S, C}_2\text{-OAc}), \ 2.40 \ (3\text{H, S, C}_7\text{-OAc}), \ 2.42 \ (3\text{H, S, C}_4\text{-OAc}), \ 2.80 \ (2\text{H, q, J = 17 Hz, C}_3\text{-2H}), \ 3.76 \ (3\text{H, S, C}_5\text{-OCH}_3). \ 5.2 \ (1\text{H, dd, J=5, 11 Hz, C}_2\text{-H}), \ 6.0 \ (1\text{H, d, J=2.5 Hz, C}_6\text{-H}), \ 6.3 \ (1\text{H, d, J=2.5 Hz, C}_8\text{-H}), \ 7.20 \ (1\text{H, S, C}_5\text{-H}), \ 7.25 \ (2\text{H, d, J=2.7 Hz, C}_2\text{-H and C}_6\text{-H}) \text{ and M}^+ \ 302 \text{ and m/e 274, 259, 167, 166, 138, 136 and 134. Alkaline degradation with (50% KOH) of the }
aglycone yielded mono-methyl ether of phloroglucinol (m.p. and Co-TLC) and Protocatechuic acid (m.p. and Co-TLC) which showed the presence of hydroxyl group at C-7, C-3, C-4' and methoxy group at C_5. position. On demethylation with (48% HBr-HOAc) it afforded eriodictyol\(^6\) (m.p. and Co-TLC). Hence the aglycone was identified as eriodictyol-5-0-methyl ether\(^7\) (Co-PC and TLC). The aglycone gave red colour with p-tolune sulphonic acid were as the glycoside did not, thereby confirming that the sugars were attached at C_7 to the aglycone in the glycoside.

On periodate oxidation the glycoside consumed 3.01 Mol of periodate with the liberation of 1.02 Mol of formic acid. This indicated the presence of a disaccharide having both units in the pyranose form. Acid hydrolysis of the permethylated glycoside yielding 2:3:6 tri-0-methyl-\(\alpha\)-galactose and 2:3:4:6, tetra-0-methyl-\(\alpha\)-glucose (Co-PC and TLC), indicating that the sugars were linked via (1→4) linkage.

Enzymatic hydrolysis of the glycoside indicated \(\beta\)-linkage between the sugar as well as between \(\alpha\)-galactose and the aglycone. Thus the glycoside was identified as eriodictyol-5-0-methyl ether 7-0-\(\beta\)-\(\alpha\)-glucopyranosyl (1→4)-3-\(\beta\)-\(\alpha\)-galactopyranoside.

Seeds of *Fagopyrum esculentum* (Moench) were supplied by M/S Himalaya-Range Drug Fields, Simla and authenticated by Botany Department of this University. The air dried and powdered
seeds were extracted with 95% ethanol and extract concentrated under reduced pressure and resolved into water soluble and water insoluble part. The concentrated water soluble portion successively extracted with Benzene, Chloroform, Ethyl acetate. The Ethyl acetate soluble part when worked up gave an amorphous mass which showed single spot on TLC (Rf 0.63, Chloroform: Methanol 3:2) it crystallized from EtOAc, petrol as yellow needles (0.067%).

ACKNOWLEDGEMENT: One of us (G.C.S.) is thankful to I.C.M.R., New Delhi for financial assistance.

REFERENCES:

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(P. F. O.)
A NEW ACYLATED QUERCETIN-3-O-β-D-GLUCOPYRANOSIDE
FROM THE LEAVES OF FAGOPYRUM CYMOSUM (MEISSM).

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Department of Chemistry,
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SAGAR (M.P.) 470.003 INDIA

Fagopyrum cymosum (Meissm)¹ (N.O. Polygonaceae) is
found from Kashmir to Sikkim. Its leaves are cooked and
eaten as vegetable, and grains are used in colic, choleriae,
diarrhoea flaxes and abdominal obstructions. Earlier workers²
have already reported the occurrence of rutin (8.5%) in
Fagopyrum species along with some poisonous substance. The
presence of a flavonoidal compound in Fagopyrum cymosum
leaves has also been reported³. It was therefore thought
worthwhile to investigate its leaves and resulted the
isolation and structure elucidation of a new acylated
flavonoid glycoside, Quercetin-3-O-(2"-O-P-coumaroyl)-β-D-
glucopyranoside.

EXPERIMENTAL :

3.0 Kg of air dried and defatted leaves of Fagopyrum
cymosum (Meissm) with 95% ethanol and the extract concentrated
to a brown viscous mass, and extracted with various solvents.
The ethyl acetate fraction when worked up by column chromato-
graphy, yielded a compound, Fc₁, which gave tests for
flavonoidal glycoside,⁴-⁵ had m.p. 259-61⁰, molecular formula,
$\text{C}_{30}\text{H}_{26}\text{O}_{14}, \text{M}^+ = 610$ (found C = 58.92%, H = 4.18%; calculated C = 59.01%, H = 4.26%). The various spectral shifts indicated the presence of free hydroxyl groups at C$_5$, C$_7$, C$_3$, C$_4$, in the FC$_1$. Its acid hydrolysis yielded Quercetin, p-coumaric acid and D-glucose (Co-PC and TLC). On treating with methanolic sodium methoxide it yielded; Quercetin-3-O-\(\beta\)-D-glucoside, m.p. 216-18°C, and methyl-p-coumarate. (Rf = 0.72) which were indentified by UV, IR, $^1$H NMR and MS analysis.

**Methyl-p-coumarate:**

$^1$H NMR (CDCl$_3$, TMS int.) $\delta$ = 7.71 ppm (d, J = 8 Hz, H-B), 7.58 (d, J = 4.5 Hz, H-2, H-6), 6.86 (d, J = 4.5 Hz, H-3, H-5), 6.30 (d, J = 8 Hz, H-), 2.48 (S-OAc), 3.82 (S,OCH$_3$). \(\nu_{\text{max}}^{\text{KBr}}\) 3400, 2980, 1725, 1675, 1600, 1550, 1320, 826 cm$^{-1}$. MS = 178 and m/e at 147, 119, 91, 69, 55.

**Quercetin-3-O-\(\beta\)-D-glucopyranoside:**

\(\lambda_{\text{max}}^{\text{MeOH}}\) 257, 279, 300 nm, (AlCl$_3$) 275, 295, 331, 438 nm, (AlCl$_3$ + HCl) 268, 299, 366, 405 nm, (NaOAc) 275, 324, 380 nm, (NaOAc + H$_3$BO$_3$) 262, 298, 377 nm, Quercetin-3-O-\(\beta\)-D-glucopyranoside permethyl ether was prepared as described by Brimacombe and worked up as usual.

**Quercetin, 5,7,3',4'-tetra-O-methyl ether:**

The permethyl ether of Quercetin-3-O-\(\beta\)-D-glucopyranoside on hydrolysis with acid resulted in the isolation of
Quercetin 5, 7, 3', 4'-tetra-O-methyl ether. Which indicated that D-glucose was attached at position C₃. \( \lambda_{\text{max}}^{\text{MeOH}} 296, 350, 296 \text{ nm}, (\text{NaOMe}) 394, 377, 271, \text{nm}, (\text{AlCl}_3) 431, 375, 333, 276 \text{ nm}, (\text{AlCl}_3 + \text{HCl}) 401, 353, 342, 272 \text{ nm}, (\text{NaOAc}) 376, 332, 272 \text{ nm}, (\text{NaOAc}) 376, 332, 272 \text{ nm}, (\text{NaOAc} + \text{H}_3\text{BO}_3) 367, 260 \text{ nm}.

Permethylation\(^7\) of \(\text{Fc}_1\) gave its octa-O-methyl-ether (II) \(\text{C}_{38}\text{H}_{42}\text{O}_{14}\), which on acidic hydrolysis yielded 5, 7, 3', 4' tetramethoxy-3-hydroxy flavone indicating that the acyl residue was attached to the glucose moiety. On acetylation with acetic anhydride and pyridine \(\text{Fc}_1\), gave an octa acetate (III), \(\text{C}_{46}\text{H}_{42}\text{O}_{22}\), m.p. 256-59°C. \(^1\)H NMR analysis of (III) showed the presence of one p-coumaroyl residue and the chemical shifts for the protons (H-1", 5.50 ppm), (H-2", 5.00 ppm) indicated acylation at position OH-2"\(^8\) thus confirming \(\text{Fc}_1\) to be Quercetin-3-O-(2"-p-coumaroyl)-\(\beta\)-D-glucoside.
which was further supported by its mass spectral analysis.

Fc₃ octa-O-acetate (III); Fc₃ were taken with acetic anhydride and pyridine and heated on a water bath for 4 hours and the acetate worked up as usual. It crystallised from MeOH:CHCl₃ 1:1, m.p. 254-56°, \( \lambda_{\text{MeOH} \text{ max}}^{\text{max}} 396, 350, 290 \) nm, (NaOMe) 394, 377, 271 nm (AlCl₃) 431, 375, 333, 276 nm, (AlCl₃/HCl) 401, 353, 376, 334, 295 nm, (NaOAc) 385, 332, 272 nm, (NaOAc/H₂BO₃) 367, 260 nm. \( \nu_{\text{KBr} \text{ max}}^{\text{max}} 3352, 1680, 1656, 1676, 1600-1550, 1284, 1220, 1140, 825. \)

\( ^1\text{H NMR}^9; \text{CDCl}_3, \text{TMS, int.} \) \( \delta = 7.35 \) (d, J = 2.5; H-2"), 7.65 (dd, J = 2.59 H-6'), 7.0 (d, J = 9.0, H-5'), 6.50 (d, J = 2.4, H-6'), 6.53 (d, J = 2.0, 7-8'), 2.38 (S, OAc-3"), 2.35 (S, OAc-4'), 2.45 (S, OAc-5), 2.49 (S, OAc-3), 4.85 (d, J = 7.8 anomeric protone), 3.75-4.30 (m; protons of sugar residue) 2.12 (S, 2" OAc), 2.14 (S, 3"-OAc), 2.10 (S, 4"-OAc), 2.08 (S, 6"-OAc). MS: \( M^+ 610 \) and m/e at 492, 465, 302, 153, 152, 150, 124.

ACKNOWLEDGEMENT:

One of us (GCS) is thankful to ICMR New Delhi for financial assistance.
REFERENCES:


* * * * *
AMINO ACID COMPOSITION OF ARTEMISIA NILEGARICA LEAVES

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Harisingh Gour Vishwavidyalaya, Sagar—480003, M.P., (INDIA)

Received: August 6, 1983: Accepted: October 3, 1983

The leaves of Artemisia nilegarica give proteins (yield 7.2%) with nitrogen contents (15.8%) consisting of seventeen amino acids. The isolated protein contains high percentage of threonine (14.82), leucine and isoleucine (12.21), glycine (12.82), glutamic acid (9.8), and arginine (8.28).

Artemisia nilegarica [1] (N. O. Compositae) is a small herb and distributed in south Africa and temperate region of north western Himalayas.

The leaves of the plant supplied by Himalaya Range Drug Field, Simila, was taken up for phytokchemical investigations because of its reputed therapeutic values. It is used for stimulating effect on the digestive organs and as a tonic.

Experimental: The proximate chemical composition of the leaves were determined by standard method (A.O.A.C.) [2], were found to contains moisture (7.1%), fat (13.2%), protein (17.24%), carbohydrates (7.61%), ash (6.3%).

The air dried and powdered leaves were extracted with petroleum ether. The defatted leaves were extracted with cold alkaline NaCl solution (15%) for fifty two hours and filtered. The filtered on acidification with 15% acetic acid precipitated protein in 17.2%.

Protein isolated was completely hydro-

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<td>16.</td>
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<td>2.46</td>
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lysed by refluxing with 6 N HCl formic acid (2 : 2 v/v) and the hydrolysate evapo-
rated to a viscous mass which was kept in a dessicator to give a residue [3].

The viscous mass was quantitatively analysed by paper chromatography [4-6] and thin layer chromatography [5] using (1) n-butanol acetic acid and water (4 : 1 : 5), (11) pyridine : water, (111) phenol : water (4 : 1) and n-butanol : pyridine : water (1 : 1 : 1) as solvent system and ninhydrin as spraying reagent.

The quantitative determination of amino acids was made from the two dimensional developed chromatographs by photometric ninhydrin method the results are given in Table 1.

Discussion: The paper and thin layer chromatography in connection with photometric estimation of ninhydrin colours revealed that the leaves of Artemisia nilegarica are rich in essential amino acids particularly lysine, threonine, leucine and isoleucine.

Acknowledgement: One of the author (A. S.) is thankful to C.S.I.R. for financial assistance.

REFERENCES

STUDIES ON ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL OF THE LEAVES OF FAGOPYRUM ESCULANTUM

SAMAIYA G.C. AND SAXENA V.K.*

Fagopyrum esculantum (Polygonaceae) is widely distributed in the temperate region of India. The plant is reported1 to be an amollicent and resolvent, and also useful in Colic, Choleic, Diarrhoea and abdominal obstructions. No antibacterial work has been previously done on the essential oil and therefore the author thought it worthwhile to isolate the essential oil from the leaves of the plant F. esculantum and study its antibacterial activity.

MATERIALS AND METHODS

The essential oil was extracted from the leaves of F. esculantum by steam distillation, using Clavengar’s apparatus. Five dilution of essential oil viz. 1 : 100, 1 : 250, 1 : 500 and 1 : 1000 were prepared using ethylene glycol as solvent and were tested against Bacillus subtilis, B. pumilus, Staphylococcus albus, S. aureas, Vibrio cholerae, Bacillus anthracis.

Bactonutrient agar and Saborand’s dextrose agar media were employed for testing the antibacterial activity, using paper disc. prepared from Whatman No. 1 filter paper. Sterilized discs. (6 mm diam.) were thoroughly soaked in pure oil and from different dilutions and placed over the seeded agar plates. The antimicrobial activity was measured2 in terms of inhibitory zone around the filter paper disc. after incubating the plates at 33°C.

**TABLE I. Antibacterial activity of the essential oil of the leaves of Fagopyrum esculantum**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Organisms</th>
<th>Diamenter of Zone of inhibition in mm.*</th>
<th>Control**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bacillus subtilis</td>
<td>18 16 17 18 17 17</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>B. pumilus</td>
<td>16 16 15 16 14 15</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Staphylococcus albus</td>
<td>15 10 11 14 13 14</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>S. aureus</td>
<td>20 18 16 19 17 18</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Vibrio cholerae</td>
<td>12 10 8 9 7 10</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Bacillus anthracis</td>
<td>14 13 12 15 11 13</td>
<td></td>
</tr>
</tbody>
</table>

* 1000 ppm griseofulvin.
** including the diameter of filter paper disc.

*Department of Chemistry, Dr. Harising Gour Vishwavidyalaya, Sagar 470 003 (M.P.)
RESULTS AND DISCUSSION

The essential oil of the leaves of *F. esculentum* showed activity (Table—1) against all the bacterias even at 1 : 1000 dilution.

ACKNOWLEDGEMENT

One of the author G.C. Samaiya is thankful to I.C.M.R. for financial assistance.

REFERENCE

STUDIES ON ANTIMICROBIAL EFFICACY OF ESSENTIAL OIL OF THE LEAVES OF ANAPHALIS CONTORTA

V. K. SAXENA, ALOK SAHAI AND GULAB SAMAIYA

Abstract

Anaphalis contorta (n.o. Compositae) is reported to stimulate appetite and is used as a general tonic. The essential oil extracted from its leaves has been found to have inhibitory activity against some human pathogenic bacteria and fungi.

Introduction

Anaphalis contorta belongs to natural order Compositae and is widely distributed in the temperate regions and Himalayas up to an elevation of 3000 m. The plant is generally used in the medicine in the form of alcoholic extracts, tinctures and medicinal wines. Taken before meals, it stimulates the appetite. It is also administered to convalescents for its sedative and tonic properties. This paper reports the effect of the oil of A. contorta as an antimicrobial agent.

Materials and Methods

The essential oil was extracted from the leaves of Anaphalis contorta by steam distillation, using Clevenger's apparatus. The different dilutions of essential oil, viz., 1:100, 1:250, 1:500 and 1:1000 were prepared using ethylene glycol as solvent and were tested against E. coli, Shigella nigroa, Sarcina lutea, Bacillus palmilus, Bacillus mycoides, Staphylococcus aureus, Microsporum gypseum, Aspergillus niger and Penicillium notatum. The antimicrobial activity was studied by filter paper disc method as suggested by Maruzzella and Henry (1958).

Bactonutrient agar and Saboraud's dextrose agar media were employed for testing the antibacterial and antifungal activity using paper disc prepared from Whatman No. 1 filter paper. Sterilized paper discs (14 mm. diam.) were thoroughly soaked in the pure essential oil and in the solutions of its different dilutions and were placed over the seeded agar plates. The antimicrobial activity was measured in terms of inhibitory zones around the filter paper disc. The zones of inhibitions were recorded after incubating the plates at 35°C in the case of bacteria and 26 °C for fungi and measurements were taken as the average of maximum dimension of the zone of inhibition in four different directions. The observations and results are recorded in Table-I.

Results and Discussion

The essential oil of the leaves of Anaphalis contorta showed maximum inhibitory activity in pure form and has inhibitory against E. coli, S. nigroa, S. aureus,
Table-1. Antibacterial and antifungal activity of the essential oil of the leaves of *Anaphalis contorta*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Organisms</th>
<th>Diameter of zone of inhibition (in mm)*</th>
<th>Control**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pure oil 1:100</td>
<td>1:250</td>
</tr>
<tr>
<td>1.</td>
<td><em>Escherichia coli</em></td>
<td>28</td>
<td>23</td>
</tr>
<tr>
<td>2.</td>
<td><em>Shigella nigoista</em></td>
<td>25</td>
<td>22.5</td>
</tr>
<tr>
<td>3.</td>
<td><em>Sarcina lutea</em></td>
<td>18</td>
<td>17.5</td>
</tr>
<tr>
<td>4.</td>
<td><em>Bacillus mycoides</em></td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>5.</td>
<td><em>B. pumilus</em></td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>6.</td>
<td><em>Staphylococcus aureus</em></td>
<td>24</td>
<td>22</td>
</tr>
<tr>
<td>7.</td>
<td><em>Aspergillus niger</em></td>
<td>25.5</td>
<td>24</td>
</tr>
<tr>
<td>8.</td>
<td><em>Penicillium notatum</em></td>
<td>19.5</td>
<td>18</td>
</tr>
</tbody>
</table>

*Including the diameter of filter paper disc

**1000 ppm griseofulvin

***Analysed statistically for the significance of inhibition

IN—Inactive

*A. niger* and *M. gypseum* even at dilutions of 1:1000.

The oil of *A. contorta* is found to inhibit a number of bacteria and fungi, including those that are pathogenic to human beings. This observation may be exploited in studying the possible role of this plant as a therapeutic agent.

**Acknowledgement**

One of the authors, Alok Sahai, is thankful to CSIR, for financial assistance.

**References**

CHEMICAL STUDY OF THE STEM OF ARTEMISIA-NILEGARICA

V. K. Saxena and G. C. Samaiya
Natural Products Laboratory
Department of Chemistry
Hari Singh Gour Vishwavidayala, Sagar—470003, (M.P.)

Received : August 18, 1983 ; Accepted : December 15, 1983

The analysis of stem of artemisia nilegarica was made by using i.r. and over techniques. The results show that it contains various sugars.

Artemisia nilegarica [1, 2, 3] (N. O. Compositae) is a small herb and is commonly found in South Africa and also occurs in the temperate region of North Western Himalayas. The stem of the plant are reported to have anthelmintic effect. The plant as well as its other species yield essential oils which are used for flavouring vinegar.

A decoction of the stem in this plant is used for stimulating the digestive organs and is also used as general tonic, because of its reputed therapeutic values.

1 kg. of the stem of Artemisia nilegarica (supplied by Himalayas range and Drug field Simla and authenticated by the Botany department of this University) was taken up for phytochemical investigations and in the present communication we report the amio acids and sugar contents of its stem.

The defatted stem was found to have 15% crude protein which on hydrolysis gave alanine, leucine, proline, cystine, aspartic acids lysine and glycine, while the water soluble part of the alcholic extract of the stem has revealed the presence of sugars identified as D-glucose, D-arabinose, D-ribose, L-rhamnose, D-galactose and raffinose, maltose, sucrose.

Experimental : The stem of Artemisia nilegarica was macerated with 10% sodium chloride solution when the protein precipitated out and was separated by filtration and hydrolysed by refluxing with 50 ml. of 6 N HCl for 18 hrs. at 105°C. The contents were decolourised by passing through animal charcoal and evaporated to dryness. The residue was dissolved in 10% isopropanol.

Paper chromatography of the amino acids was carried out on Whatman filter paper No. 1 of (16 x 20") size. The chromatography chamber was saturated with solvent (ii) n-butanol containing 3% NH₄OH and (iii) tertiarly amylalcohol. The dried Whatman systems (i) phenol containing 3% NH₄OH no. 1 paper was spotted with the test and authentic amino acids about 1.0 cm. apart from each other and about 1 cm. below the
<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Phenol containing 3% NH₃</th>
<th>n-butanol; Alcohol containing 3% NH₃</th>
<th>Tertiary aminalcohol</th>
<th>Amino acids identified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RₛΨ Reported</td>
<td>RₛΨ Found</td>
<td>RₛΨ Reported</td>
</tr>
<tr>
<td>1.</td>
<td>0.54</td>
<td>0.52</td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>2.</td>
<td>0.83</td>
<td>0.82</td>
<td></td>
<td>0.46</td>
</tr>
<tr>
<td>3.</td>
<td>0.85</td>
<td>0.84</td>
<td></td>
<td>0.14</td>
</tr>
<tr>
<td>4.</td>
<td>0.24</td>
<td>0.22</td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>5.</td>
<td>0.12</td>
<td>0.11</td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>6.</td>
<td>0.46</td>
<td>0.45</td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>7.</td>
<td>0.40</td>
<td>0.39</td>
<td></td>
<td>0.05</td>
</tr>
</tbody>
</table>

edge of the filter paper. The paper was dried for 24 hours and were developed by spraying with ninhydrin (0.2% in acetone) followed by heated in an electric oven for 10 minutes at 60°C. The sets of authentic amino acids having wide difference of RₛΨ values were spotted with unknown solution on other Whatman filter paper No. 1.

The RₛΨ values of unknown and authentic amino acids as obtained are given in Table 1.

**PART II**

The defatted stem were extracted with rectified spirit and the extract concentrated to a green brown syrupy mass dissolved in distilled water (1000 ml.)

The water soluble part was concentrated and was found to give positive Molish's test indicating the presence of free sugars. The identity of the sugars was confirmed by paper chromatography using (i) n-butanol, glacial acetic acid : water (4 : 1 : 5) and (ii) n-butanol : ethanol : water : amonia (45 : 5 : 48 : 2) system and aniline hydrogen phthalate as spraying reagent when green brown spot appeared on the paper. The observation and results are recorded in Table 2.

The presence of these amino acids and sugar was further confirmed by thin layer chromatography. Silica gel was deposited on the glass plates of (20 x 20 cm) size and the plates activated by keeping in the electric oven at 70°C.

The plates were developed with the solvent system (i) benzene : glacial acetic acid : methanol (20 : 20 : 60) (ii) n-butanol : glacial acetic acid : water (60 : 30 : 10) for sugar and 96% ethanol : water (60 : 40) and n-butanol : glacial acetic acid : water, (6 : 2 : 2) for amino acids and sprayed with aniline hydrogen phthalate and ninhydrin respectively. The observation and results are recorded in the Table 3. Sugar and (IV) amino acid and further confirmed by the result as derived from paper chromatography.
### TABLE 2

**SOLVENT SYSTEM**

<table>
<thead>
<tr>
<th>No.</th>
<th>n-butanol : acetic acid : water (4:1:5)</th>
<th>n-butanol : ethanol water : ammonia 1% (45:5:49:1)</th>
<th>Sugar Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R₁ Reported R₁ Found</td>
<td>R₁ Reported R₁ Found</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>0.18 0.17</td>
<td>0.105 0.102</td>
<td>D-glucose</td>
</tr>
<tr>
<td>2.</td>
<td>0.21 0.20</td>
<td>0.145 0.146</td>
<td>D-arabinose</td>
</tr>
<tr>
<td>3.</td>
<td>0.31 0.29</td>
<td>0.110 0.299</td>
<td>D-ribose</td>
</tr>
<tr>
<td>4.</td>
<td>0.37 0.34</td>
<td>0.285 0.276</td>
<td>L-rhamnose</td>
</tr>
<tr>
<td>5.</td>
<td>0.05 0.04</td>
<td>—     —</td>
<td>Raffinose</td>
</tr>
<tr>
<td>6.</td>
<td>1.16 0.14</td>
<td>0.09 0.08</td>
<td>D-galactose</td>
</tr>
<tr>
<td>7.</td>
<td>0.11 0.10</td>
<td>0.15 0.14</td>
<td>Maltose</td>
</tr>
<tr>
<td>8.</td>
<td>0.10 0.13</td>
<td>—     —</td>
<td>Sucrose</td>
</tr>
</tbody>
</table>

### TABLE 3

**SOLVENT SYSTEM**

<table>
<thead>
<tr>
<th>No.</th>
<th>Benzene : glacial acetic acid : methanol (20:20:60)</th>
<th>n-butanol : glacial acetic acid : water (60:30:10)</th>
<th>Sugar Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R₁×100 Reported R₁×100 Found</td>
<td>R₁×100 Reported R₁×100 Found</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>63 64</td>
<td>39 37</td>
<td>D-glucose</td>
</tr>
<tr>
<td>2.</td>
<td>62 60</td>
<td>43 41</td>
<td>D-arabinose</td>
</tr>
<tr>
<td>3.</td>
<td>—         —</td>
<td>—         —</td>
<td>D-ribose</td>
</tr>
<tr>
<td>4.</td>
<td>57 59</td>
<td>59 60</td>
<td>L-rhamnose</td>
</tr>
<tr>
<td>5.</td>
<td>—         —</td>
<td>20 18</td>
<td>Raffinose</td>
</tr>
<tr>
<td>6.</td>
<td>55 53</td>
<td>37 35</td>
<td>D-galactose</td>
</tr>
<tr>
<td>7.</td>
<td>—         —</td>
<td>—         —</td>
<td>L-ascorbic acid</td>
</tr>
<tr>
<td>Sl. No.</td>
<td>95% ethanol : water (60 : 40)</td>
<td>n-butanol : glacial acetic acid : water (6 : 2 : 2)</td>
<td>Amino acid Identified</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------</td>
<td>--------------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td></td>
<td>R_f × 100</td>
<td>R_f × 100</td>
<td>R_f × 100</td>
</tr>
<tr>
<td></td>
<td>Reported</td>
<td>Found</td>
<td>Reported</td>
</tr>
<tr>
<td>1.</td>
<td>47</td>
<td>46</td>
<td>22</td>
</tr>
<tr>
<td>2.</td>
<td>—</td>
<td>—</td>
<td>44</td>
</tr>
<tr>
<td>3.</td>
<td>61</td>
<td>59</td>
<td>14</td>
</tr>
<tr>
<td>4.</td>
<td>35</td>
<td>33</td>
<td>09</td>
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<td>5.</td>
<td>39</td>
<td>38</td>
<td>17</td>
</tr>
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<td>6.</td>
<td>43</td>
<td>45</td>
<td>18</td>
</tr>
<tr>
<td>7.</td>
<td>03</td>
<td>04</td>
<td>03</td>
</tr>
</tbody>
</table>

REFERENCES

Essential Oil Association Of India
H. B. T. I., CAMPUS, P. O. NAWABGANJ, KANPUR-208 002. (INDIA)

Ref. No. .................
EOAI/HO/Pub-IP/ 143 /85

Date........5th March 85

Dear Sir,

This is to acknowledge the receipt of your letter No. Nil dated 21.2.85 enclosing the manuscript of your article entitled "T.L.C. AND G.L.C. STUDIES OF THE ESSENTIAL OIL FROM FAGOPYRUM TATARICUM LEAVES" for publication in the Indian Perfumer of the Association. A decision regarding its publication will be taken soon and you will be informed accordingly. For further correspondence please mention the above reference number and date.

While thanking you for your esteemed cooperation,

Yours faithfully,

(S N Kapoor)
HONY SECRETARY

TO

Dr. V.K. Saxena

14, Ashok Road, Cantonment,

SAGAR (M.P.)
T.L.C. AND G.L.C. STUDIES OF THE ESSENTIAL OIL FROM
FAGOPYRUM TATARICUM LEAVES

By
SAMAIYA, G.C. AND SAXENA, V.K.

Department of Chemistry, Dr. Harisingh Gour Vishwavidyalaya,
SAGAR(N.P.) INDIA.

ABSTRACT

Fagopyrum tataricum Gaertn which occurs in colder part of Laddakh is reported to be useful in Colic, Cholic, Diarrhoea and Abdominal Obstructions.

The leaves of Fagopyrum tataricum on steam distillation yielded an essential oil in an yield of 0.36% which on T.L.C. and G.L.C. examinations was found to consist of the following components in the percentage shown against each; P. cymene 6.72%; α-pinene, 8.76%; d-Limonene, 8.60%; α-terpenol 15.8%; Bornyl acetate 17.3%; α-thujene 14.0%; α-terpenoline 2.0%; Myrcine 7.1%; camphene 5.1%; Methyl chavicol 9.2%; eugenol 2.5% and unidentified alcohol 2.2% and unidentified constituents 0.56%.

EXPERIMENTAL

Fagopyrum tataricum Gaertn (N.O. Polygonaceae) is cultivated in the higher Himalayans at an altitudes of 3000-15,000 ft., and occurs especially in the colder parts of Laddakh at Zaskre and Western Tibet. It is reported to be used in colic, cholecic, diarrhoea and abdominal obstructions.
Essential oil was obtained by steam distillation from fresh leaves of *Fagonyrum tataricum*. The authentic samples of pure constituents of essential oils were supplied through the courtesy of M/S Fritz Brothers, Inc. New York, U.S.A.

**T.L.C. ANALYSIS**

Silica gel G (Merck) was spread over glass plate as 0.20 mm thin layer. The T.L.C. plates were prepared by uniformly spreading a slurry of Silica gel G in distilled water, and the plates activated by usual procedure. Samples of essential oil 5 µg were applied 2-5 cm. above the lower edge of the plates and developed in a solvent system.

(I) Hexane, (II) 2-2 dimethyl butane

(III) Benzene (IV) Methyl hexane.

For visualising the separated components, vanillin sulphuric acid was used as spraying reagent, then the plates were heated at 105°C for 10 minutes in an electric oven. The results obtained are summarised in Table I.

**G.L.C. ANALYSIS**

For G.L.C. different packing material e.g. 56%; OV-17 and SE 30 and different working conditions were tried and best results were obtained under the following conditions:

Technique - Linear temperature programming 62-200°C increased by 2°C per minute.
Column - Coiled glass column, 145 cm long and 0.24 mm in diameter; packed with 3Y OV 17.

Detector - Flame ionisation detector
Attenuation = $4 \times 10^4$
Carrier gas - Nitrogen
Pressure of carrier gas = 1.5 Kg/cm$^2$
Injection temperature - 168°C
Detector temperature - 220°C
Chart speed - 11"/hour

Identification of the various constituents was possible by comparing the retention. Values at different temperatures at which their peaks appeared with those of the pure substances as well as by adding the authenticity to the oil before injection and observing the increase in their corresponding peaks.

The quantitative determination was done from the values automatically integrated for peak areas. The results obtained are compiled in Table II and shown in Figure 2.

From the quantitative determination it is evident that bornyl acetate (17.3%) is the major constituent followed by $\alpha$-terpenol (15.8%) and $\alpha$-Thujene (14.8%).
### TABLE I

RESULTS OF T.L.C. ANALYSIS OF PURE AUTHENTICS IN DIFFERENT SOLVENT SYSTEMS

<table>
<thead>
<tr>
<th>Compound</th>
<th>Colour</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-cymene</td>
<td>Light green</td>
<td>34</td>
<td>42</td>
<td>54</td>
<td>58</td>
</tr>
<tr>
<td>(\alpha)-pinene</td>
<td>Light blue</td>
<td>64</td>
<td>68</td>
<td>70</td>
<td>78</td>
</tr>
<tr>
<td>d-Limonen</td>
<td>Light blue</td>
<td>48</td>
<td>58</td>
<td>56</td>
<td>74</td>
</tr>
<tr>
<td>(\alpha)-Thujene</td>
<td>Violet</td>
<td>66</td>
<td>72</td>
<td>80</td>
<td>84</td>
</tr>
<tr>
<td>(\alpha)-Terpenol</td>
<td>Greenish blue</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bormyl acetate</td>
<td>Brown blue</td>
<td>58</td>
<td>64</td>
<td>66</td>
<td>70</td>
</tr>
<tr>
<td>(\alpha)-Terpenolene</td>
<td>Blue</td>
<td>66</td>
<td>67</td>
<td>66</td>
<td>66</td>
</tr>
<tr>
<td>Methyl chavicol</td>
<td>Brown blue</td>
<td>-</td>
<td>-</td>
<td>60</td>
<td>-</td>
</tr>
<tr>
<td>Camphene</td>
<td></td>
<td>80</td>
<td>76</td>
<td>78</td>
<td>78</td>
</tr>
<tr>
<td>Eugenol</td>
<td>Violet brown</td>
<td>14</td>
<td>34</td>
<td>28</td>
<td>-</td>
</tr>
<tr>
<td>Peak No.</td>
<td>Corresponding authentic</td>
<td>Temp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-------------------------</td>
<td>------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>α-Pinene</td>
<td>68</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>P-cymene</td>
<td>68</td>
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</tr>
<tr>
<td>3.</td>
<td>d-Limonene</td>
<td>98</td>
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</tr>
<tr>
<td>4.</td>
<td>α-Thujene</td>
<td>110</td>
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<td>α-Terpenol</td>
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<tr>
<td>6.</td>
<td>Bornyl acetate</td>
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<tr>
<td>7.</td>
<td>α-Terpenolene</td>
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</tr>
<tr>
<td>8.</td>
<td>Murenone</td>
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REFERENCES


GAS CHROMATOGRAM OF E.OIL OF LEAVES OF FAGOPYRUM TATARICUM
A NEW FLAVONE RUTINOSIDE FROM FAGOPYRUM TATARICUM

(Quercetin 3', 4', 5, 7-tetramethyl ether-3-O-β-D-
rutinoside/Fagopyrum tataricum)

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ABSTRACT: A new flavone glycoside, quercetin 3', 4', 5, 7-
tetramethyl-3-O-β-rutinoside has been isolated from Fagopyrum
Tataricum leaves.

Fagopyrum tataricum Gaertn1,2 (N.O. Polygonaceae) is
commonly used in colic, cholerae, diarrhoea, flaxes and
abdominal obstructions. Earlier workers have already reported
the occurrence of rutin (8.5%)3 in Fagopyrum species along with
some poisonous substance. The presence of a flavonoidal
compound in Fagopyrum tataricum leaves have also been reported4.
Its methanol soluble fraction of leaves was, therefore, worked
up and a new flavone glycoside, quercetin 3', 4', 5, 7-tetramethyl
ether-3-O-β-D-rutinoside was obtained.

From the concentrated methanolic leaves extract of
Fagopyrum tataricum, a new flavone glycoside A was isolated.
It analysed for molecular formula C31H38O16 m.p. 179-180°C,
M+ 666 and gave positive test of flavone glycoside5,6 and showed