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New Natural Products from Medicinal Plants with Special Reference to Novel Flavonoids, Biflavonoid & Their Biological Implications

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ABSTRACT

The aim of the research is to isolate and characterize the new flavonoids and biflavonoids from medicinal plants and to check their biological activity including Vitamin P activity; diuretic action; antibacterial activity; prophylactic actions; estrogenic activity and free radical scavenging activities. The present paper deals with isolation of many novel & rare bioactive flavonoids & biflavonoids isolated for the first time from plants. Isolation and characterization of these flavonoids have been carried out using modern techniques. Pharmacological & toxicological studies have been carried out using animal models. The complex mixture of biflavonoids in the leaves extract of *Taiwania cryptomerioides* have been identified as hinokiflavone, isocryptomerin, amentoflavone, sequoiaflavone, 1-7, 11-7-di-o-methyl amentoflavone, and a new series of (I-3',11-3) linked biflavone. As it constitutes the first report of the occurrence, isolation & characterization of (I-3'-11-3) biapegeninyl from Taiwania cryptomerioides, the parent biflavone has been named as Taiwaniaflavone. The structures of the parent compounds, acetates & methyl ethers were confirmed using UV, IH-NMR and mass spectrometric techniques -The isolation & structure determination of many novel flavonoids & biflavonoids , to name a few : Swertisin 6"-O-rhamnoside from *Fagraea obovata* Wall, first report of Biflavones from Manihot utilissima, a new biflavone from Ochna pumilla Ham., biflavonoid pigments from the leaves of Cupressus lawsoniana and. Fitzrova patagonica Hook., Sorbifolin-6galactoside from Garcinia andamanica King, biflavones from the genus Podocarpus, will be discussed along with biological activities for many of them. It has been found that these flavonoids have a remarkable range of activities.

KEYWORDS

Medicinal plants; Isolation; Characterization; Spectroscopic studies; Chromatographic techniques; pharmacological studies; Biological implications, Natural Products, Flavonoids, Biflavonoids

INTRODUCTION

The plant kingdom offers a rich source of structural biodiversity in the form of a variety of Natural Products. As we know, natural products continue to play an important role especially in pharmaceutical & food industries. Flavonoid covers a large group of naturally occurring, low molecular phenolic compounds found practically in all parts of the plant. Over several decades

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of research, it has been found that a variety of flavonoids have a remarkable range of activities.

Aim

Looking into the broad spectrum of biological activities within the group and the multiplicity of actions displayed by certain individual members make these flavonoids as one of the most intriguing class of biologically active compounds. The aim of the research is to isolate and characterize the homogeneous flavonoids and bioflavonoids from medicinal plants and to check their biological activity including Vitamin P activity; diuretic action; antibacterial activity; prophylactic actions; estrogenic activity and free radical scavenging activities. The present paper deals with isolation of many novel & rare bioactive flavonoids & bioflavonoids isolated for the first time from plants, their characterization using recent techniques and checking their biological activities.

Methodology

For identification purpose the extraction have been carried out using Buchi Rota vapor, isolation and characterization of these flavonoids are carried out using high speed counter current and thin layer chromatography whereas, for characterization Nuclear magnetic resonance spectroscopy and Mass spectrometry have been used besides UV, IR and GCMS methods. PharmacologicaI & toxicological studies have been carried out in bio medical department having all modern techniques and using recent models.

Result

To name a few Taiwania flavone series have been isolated from Taiwania cryptomeriodes Hayata Figure 1, Swertisin.

6"- O-rhamnoside from Fagraea obovata Wall, Figure 2. First report of Biflavones from Manihot utilissima, a new biflavone from Ochna pumilla Ham. , biflavonoid pigments from the leaves of Cupressus lawsoniana And. Fitzrova patagonica Hook, Sorbifolin-6galactoside from Garcinia andamanica King. , biflavones from the genus Podocarpus., di-Hydroxy Wogonin 7-0-cc neohesperidoside from Garcinia andamanica, a new biflavone from Ochna pumila Ham. , Mesuein: A novel flavanone glycoside from Mesua ferrea Linn. , a new glycosyl flavone from Fagraea obovata Wall. ,). Semecarpetin: a new flavone glycoside from Semecarpus Kurzii Engler., Setaricin: a new flavone glycoside from Setarica italica (Linn.) Beauv., two new flavonol glycosides from Chenopodium ambrosiodes., the isolation and characterization of Leuteolin-6-0ß-Dglucopyranoside-3-O-X-rhamnopyranoside from Ficus infectoria, 8,3 Dimethoxy -5, 4-dihydroxy Flavone, 7-0-glucoside: a new flavone glycoside from Setaria italica, flavonoidic constituents of Quercus infectoria, Scutellarein6-0-(1 -L-rhamnopyramosyl (1-2)-ß-D-galactopyranoside: two new flavone diglycoside from Ficus infectoria., isolation and characterization of two new flavanone disaccharides from the leaves of *Tecoma grandiflora*.

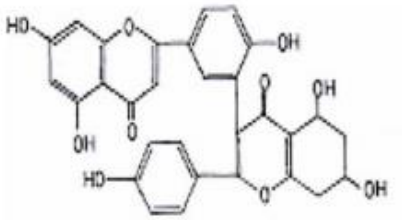

Figure 1: Swertisin 6"- O-rhamnoside.

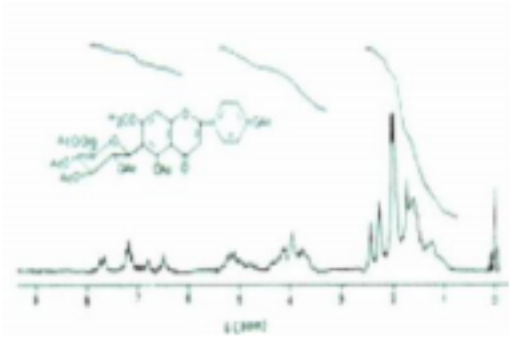

Figure 2: Biflavones from Manihot utilissima**.**

Isolation and identification of a flavonol glycoside using high speed counter current chromatographic technique from the leaves of Salvadora persica. Results of separation

of a crude flavonoid sample, separated and identified as Kaempferol 3-ct-L-rhamnosyl-7-ßxylopyranoside 1 along with Quercetin and Kaempferol from the 10% EtOH extract of the leaves of Salvadora persica through centrifugal partition chromatography (Pharma-Tech CCC-1000 Instrument) using upper phase as mobile phase and solvent system CHCIS, MeOH; EtOH; 1-120 (5:3:3:4). Detection was carried out at 254nm with flow rate 3ml / min and a rotational speed 800rpm Kamil et al. 2000 [5].

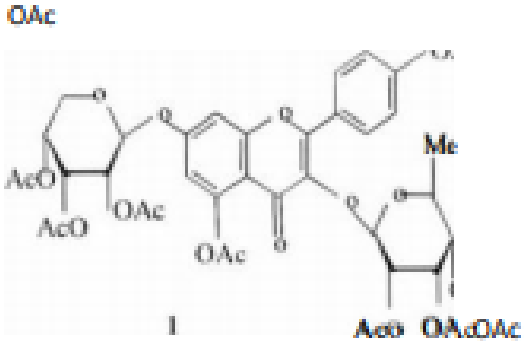

Figure 3: Three flavonoid peaks were resolved and eluted out in 3.5hrs.

The results obtained with the present CCC method are substantially far better than those from the conventional Column chromatography in terms of peak resolution and separation time; furthermore, separations are much improved. *Salvadora persica L (Salvadoraceae)* commonly known as toothbrush tree in the Middle East, is widely cultivated in China, India, Persia, Middle East and Southern Africa. Different parts of the plants are used for treating swellings, ulcers and blisters, scorpion stings and for regulating menstruation Ghazanfar 1994 [1]. Earlier report of Malik et al. 1987 [7] shows the presence of an alkaloid only. We now wish to report the separation and identification of flavonoids including a rare flavonol glycoside Neeru et al. 1990 [9] from the leaves of *Salvadora persica*. HSCCC: Counter Current Chromatography (CCC) is an efficient technique of partition chromatography that totally eliminates the use of solid supports. The name was derived from two classical partition methods: counter current distribution (CCD) and liquid chromatography (LC). These instruments are especially suitable for products in both analytical and preparative purposes. High-speed counter current chromatography (HSCCC) is an advanced design of counter current chromatography that uses centrifugal forces to achieve high retention of the stationary phase and vigorous mixing between the stationary and mobile phases as the mobile phase elutes through a series of columns. The operating principles of HSCCC are based on the hydrodynamic equilibrium system (HIDES) which was defined by Ito 1996 [3].

EXPERIMENT

Mps; uncorrected UV spectra were run in MeOH and IR spectra in KBr disc. I HNMR spectra were run at 400 MHZ. Chemical shifts are given in (ppm) with TMS as internal Standard. TLC was performed using silica gel 60F254 (Merck). What man No. 1 paper was used in paper chromatography (PC)? The TLC spots were visualized in UV light separating natural (365nm), after spraying with natural products-polyethylene glycol reagent (NP/PEG). For HSCCC two phase solvent system, composed of chloroform: methanol: ethanol water at a 5:3:3:4 volume ratios were exclusively used. Yellowish brown plant extract used for CCC separation was obtained from dried *Salvadora persica* leaves by 10% ethanol Me Aco OAc extraction. The final solution was prepared by dissolving the above extract in the solvent mixture at a concentration of 2%. The yield of 1 was found (0.57%) with respect to 10% ethanol extract. 4.1.1.1 Acid Hydrolysis of 1 The glycoside was refluxed in 2M HCI -MeOH (5ml), for 2 hr., then H20 was added and the mixture was extracted with EtOAC. The aqueous layer was neutralized with Ag2C03, the ppt. filtered and the filtrate evaporated to give a residue. The aglycone in the EtOAC fraction was crystallized from CHCl3 MeOH as yellow needles, m.p. 280-2810 and identified as Kampferol by spectral and chromatographic comparison with an authentic sample.

Acetylation

Compound 1 was acetylated with AC20 and pyridine (I:I) at room temperature for 48 hours and worked up in usual way. I HNMR (400MHz, CHC13) 87.82, (2H, d J=9Hz, H-2-,6- 7.30 (2H,d, J=9Hz, H-3-.5-), 7.04 (IH,d, 2.4Hz, H-6), 5.19 (IH,d, J=8.3Hz, H-1 xyl), 5.59 OH, d, J=1Hz, El-I-Rham); 1.22 (311, d, J=6.1Hz, Rham-Me), 3.75-5.70 (1 OH, m, Gly-H), 2.2. (3H, s, OAc-5) 2.28 (3H, s, OAc-4-) 1.98-2.20 (18 H, m, aliphatic MeCO).

PROCEDURE

Separation of crude flavonoid sample was performed by HSCCC instrument using the upper phase as the mobile phase. Separation with multilayer coil plant centrifuge was performed as follows: The coiled column was first filled with stationary lower phase followed by injection of sample solution through the sample port. Then, the apparatus was rotated at the optimum revolutional speed 800 rpm while the upper mobile phase was pumped into the head and of the column at 200 ml /hr. flow rate. Effluent from the tail of the coiled column was continuously monitored with a UV detector at 254nm and fractionated into test tubes with a fraction collector. Aliquot of each fraction was diluted with methanol and the absorbance was determined at 254nm with spectrophotometer.

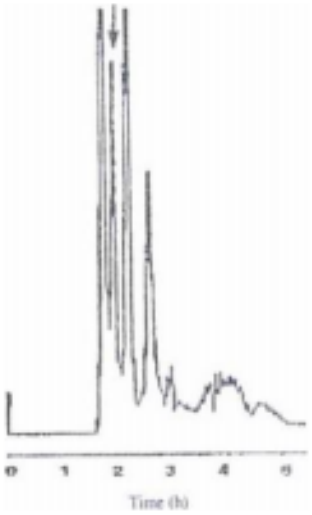

Figure 4: Separation of a flavonol glycoside from Salvadora persica by CPC.

RESULTS AND DISCUSSION

Compound 1 analysed for C26H28014. Its IR spectrum showed strong absorption bands at 3420 (OH), 1655 (C=O), 2950 (C-H), 1620 (C=c, aromatic) and a broad band at 1120-1000cm-l (C-O). Colour reactions (dull ochre to fluorescent yellow in UV + NH3), Rf values and UV spectral data with diagnostic shift reagents Mabry et al. 1970[6] and Harborne et al. 1975[2] suggested it to be a 3,4-di substituted flavonol glycoside with free hydroxyl groups at the C-5 and C-4 positions on a 3,7-disubstituted flavonol glycoside frame work. Total acid hydrolysis of 1 with 2M-HCl yielded an equimolar mixture of L-rhamnose, D xylose (PC and GLC) and Kaempferol (spectral and chromatographic comparison. Enzymatic hydrolysis of 1 with ß-xylosidase gave the same products as above. However, a bathochromic shift of 12nm in band IL with NaOAC (absent in glycoside) indicated that D-xylose was attached at the C-7 position in 1 Kamil et al. 1990[9]. On the basis of above findings, 1 was identified as Kaempferol 3-(1-rhamnopyranoside 7-ß-xylopyranoside, a rare natural product being reported for the first time from Salvadora persica. The constituent's quercetin and kaempferol were identified by standard procedures and direct comparison with authentic samples. 4.2 A new series of [I-3', II-3 linked biflavone from Taiwania cryptomerioides: Taiwania cryptomerioides Hayata belonging to the family taxodiaceae, a remarkable conifer resembles Cryptomeria in habit, and appears to be closely allied to Cunninghamia in the structure of the cone.lt was discovered in 1904 on Mount Morrison, Formosa. The present paper deals with the study of the complex mixture of biflavonoidsin the leaves extract of Taiwania cryptomerioides as hinokiflavone, isocryptomerin,amentoflavone, sequoiaflavone, 1-7, 11-7-di-o-methyl amentoflavone, and a new series of [I-3',II-3] linked biflavone. As it constitutes the first report of the occurrence, isolation & characterization of [I-3'-II-3] biapegeninyl from Taiwania cryptomerioides, the parent

biflavone has been named as Taiwania flavone Fig. (Kamil 1981).

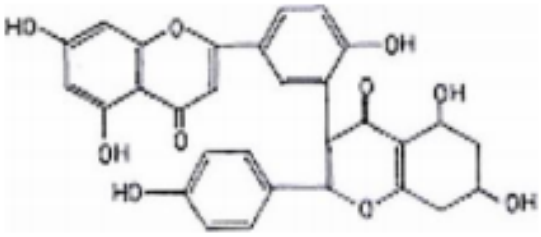

Figure 5: Taiwania flavone.

The new series consists of the parent, mono-and dimethyl ethers of biflavones as 1-4',I-5,II-5,I-7,II-7-Hexa hydroxy [1- 3', II-3] biflavone; 1-4',II-4',I-5,II-5,1-7 0r 11-7-Pentahydroxy-1-7 or 11-7-0-methyl [I-3',II-3] biflavone and 1-4',I-7,II7 tetra hydroxy-11-4',1-7/11-7-di-O-methyl [1-3',II-3] biflavone. The structures of the parent compounds, acetates & methyl ethers were confirmed using UV, IH-NMR and mass spectrometric techniques.

Setaricin- A new flavones glycoside from Setaria italica

Setaria italica Beauv has wide distribution in India, China, Japan, South Africa, South and East Europe, and North America. The methanol extract of the air- dried leaves of *Setaria italica*, after preliminary purification, was separated by column chromatography over silica gel. The chloroform-methanol (9:1) eluate was subjected to repeated column chromatography followed by preparative TLC (C6H6-MeOH-AcOH, 45:14:6) to give the new glycoside Setaricin. It was crystallized from methanol as shinning yellow needles; mp Found requires C 57.34; H 4.86, C22 H22 O11 requiresC57.14; H, 4.76 percent). Acid hydrolysis of the glycoside with0.2N hydrochloric acid gave arabinose (PC and GLC) and an aglycone (Il) which showed a bathochromic shift of +12 nm in sodium acetate(absent in glycoside) thus showing that the sugar is linked to the 7-position of the aglycone. The aglycone was characterized as 5,7,4'-trihydroxy-3',5'-dimethoxy flavones (tricin), m.p. 291-3°C by spectral and chromatographic comparison with an authentic sample (Found C,61.98; H, 4.33. calc. for C17H140; C,61.81; H.4.24 percent).

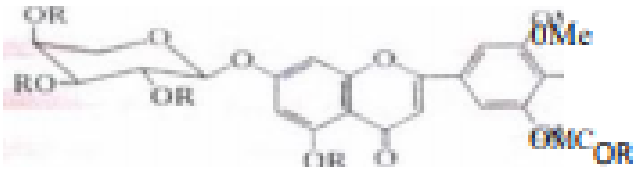

Figure 6: 5,7,4'-trihydroxy-3',5'-dimethoxy flavones (tricin).

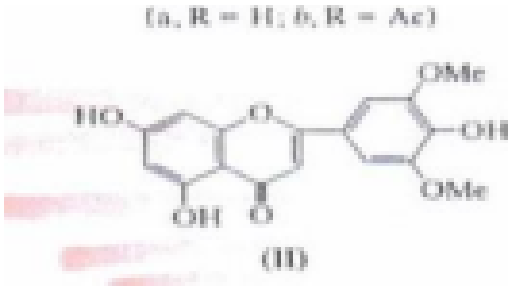

Figure 7: Acetylation of the glycoside (AC20-Py) afforded pent acetate (1b).

Acetylation of the glycoside (AC20-Py) afforded a pent acetate (1b), mp 128-300c in CDC13 showed two phenolic acetoxyls at S2.44 (3H) and 2.37 (3H) and three sugar acetoxyls in the range $2.05-2.17 (9H). The two Meta coupled protons at C (6) and C (8) appeared at $6.70 and 7.02 (J 2 1-IZ each), while a C3 proton resonated as a singlet at $ 7.05 typical for a symmetrically substituted myricetin type B-ring. The anomeric proton (-1") of arabinose appeared as a doublet at $ 5.33(J 8 Hz). The chemical shift confirm the direct attachment of sugar to the aglycone and the diaxial coupling (J 8Hz between (l") and H (-2") suggested the beta configuration of L-arabinose. The presence of peaks at m/z 259,199,169,157 and 130 in the mass spectrum of the acetate (1b) finally established the sugar moiety as a pentopyranose. The mass spectrum of the glycoside (la) fully supported the assigned structure of the glycoside as it exhibited M+ at 330 (M-gly) as the base peak. This was further supported by RDA fragmentation representing rings A with two hydroxyl & two methyl one-hydroxyl groups in ring B of

the aglycone (Il). Kuhn methylation (Mel/DMF/Ag20) of compound (la) followed by acid hydrolysis (0.3N HCL,4h, reflux temperature) gave 2,3,4-tri -O-methyl-L arabinose as a colorless liquid (alpha m.p.-1220 (in water), found OMe 48.9; calc. for C8H1605; 48.5 percent) from which crystalline 2,3,4-tri-O- methyl-Larabonic acid (mp 156 o c) 2,3,4-tri-O- methyl-Larabino phenyl hydrozidine (mp 1600C) were prepared. Also, from the hydrolysis, an aglycone was obtained which was characterized as 7-hydroxy-5,3',4',5'-tetra methoxy flavone. It showed a bathochromic shift of + I nm with sodium acetate, thus finally confirming the sugar residue at position Neeru et al. 1989 [8]. On the basis of these results, the glycoside was identified as tricin-7-O-Larabinoside and named as Setaricin. It is interesting to note that the configuration that the configuration of the L-arabinopyranose is beta, which is of rare occurrence. As far as I am aware, this novel flavone glycoside is being reported for the first time. In addition, this constitutes the first report of any flavones glycoside from this plant and appears to be the only second report of tricin glycoside from the family Graminaceae. 0Me OMC Biological & Pharmacological functions of flavonoids are many & varied The Flavonoids, 1988 [11]. The biological functions of flavonoids in man & animal was first suggested by Szent-Gyorgyi who reported that the flavonoids present in citrus peels were effective in preventing capillary bleeding & fragility associated with scurvy. Rusznyak & Szent Gyorgi provided a sample of impure ascorbic acid to a physician for administration to a patient suffering from subcutaneous capillary bleeding. The patient was cured, later they provided a more purified preparation which had no effect on other patients suffering from same malady. Returning to their impure fractions, they found contaminants with characteristics of flavones in preparations from fruits of both *Capsicum annum (Solanaceae)* & *Citrus lemon (Rutaceae).* Laboratory experiments showed that periodic intravenous Injections of these biflavonoids restored normal capillary resistance in a fortnight. They called this preparation "VITAMIN-P" and claimed that it reduced hemorrhages, extended the effects of ascorbic acid and reduced Vascular purpura. The pharmacological and biological activities of novel compounds dealt above are under active progress.

REFERENCES

1. Ghazanfar AS (1994) A handbook of medicinal plants. CRC Press, Boca Rota, Florida 33431: 190-192.
2. Harborne JB, Mabry TJ, Mabry H (1975) 'The Flavonoids'. Chapman and Hall, London 48-63.
3. Ito Y, Conway WD (1996) High-speed counter current chromatography. John Wiley and Sons, New York, USA 5-42.
4. Kamil M, Ilyas M, Rahman W (1981) Biflavanoids from the leaves of Taiwania cryptomerioides Hayata (Toxodiaceae). J.C.S. Perkin I (London) 553-559.
5. Kamil M, Ahmad FA, Jairaj AF, et al. (2000) Isolation and characterization of a flavonol glycoside using high speed counter chromatographic technique from the leaves of *Salvodora persica*. Pakistan Journal of Scientific Research 13(4): 255-257.
6. Mabry TJ, Markham KR, Thomas MB (1970) 'The Systematic identification of flavonoids'. Springer-Verlag Berlin Heidelberg 10(2): 320-320.
7. Malik S, Ahmad SS, Haider SI, et al. (1987) Salvadorine- A new indole alkaloid from the leaves of *Salvadora persica*. Tetrahedron Letters 28(2): 163-164.
8. Neeru J, MS Alam, Kamil M, et al. (1989) Setaricin: A new flavones glycoside from *Setaria italica*. Chemistry & Industry (London) 13: 422-423.

9. Jain N, Alam MS, Kamil M, et al. (1990) Two new flavonol glycosides from *Chenopodium ambrosioides*.19th World Congress Acupuncture and Complementary Medicines Frementle, Australia.
10. Jain N, Alam MS, Kamil M, et al. (1990) Two flavonol glycosides from Chenopodium ambrosioides. Phytochemistry 29(12): 3988-3991.
11. Harborne JB (1988) The Flavonoids, Advances in research since 1980. Springer, New York.