International Scholars Journals

African Journal of Internal Medicine ISSN 2326-7283 Vol. 9 (2), pp. 001-006, February, 2021. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Isolation and identification of new eleven constituents from medicinal plant

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Accepted 21 September, 2020

In order to know the appropriate medicinal plants compound we estimate oils those are complex mixtures of numerous components. The valuation of an essential oil basing only on the percentage of the main physicochemical component constants. Analyses in Semnan province revealed the presence of sesquiterpene lactones, which a group of compounds previously known for their significant bioactivity. Semnan University laboratories have succeeded in isolating and identifying 11 new constituents from a class of compounds called the steroid glucosides. High concentrations of quercetin, quercetin 4^{I}-glucoside, taxifolin, taxifolin 7-glucoside and phenylalanine were also isolated from the red bulbs of *Allium cepa* L. var. Tropea. This new discipline has grown out of the scientific endeavor to understand more about the ways in which animals may be treating themselves with the use of plants. Due to the constraints and difficulties of systematic research of this kind on wild animal populations, our knowledge is still limited. Our results suggest that organ for synthesis site of glucosides is leaves not roots of the whole plants. We have established that traditional medicinal plants from Iran contain some medicinal chemical compound by its root, leaves, flowers and shoot cultures.

Key words: Qualitative analysis, medicinal compounds, oil plant, glucosides.

INTRODUCTION

Medicinal plants are valuable sources of medicinal and many other pharmaceutical Natural products. It has been used for medicinal purposes since ancient times. These medicinal materials include products from plant, animal and mineral sources. Majority are derived from plants. Traditions usage of those material differ among various cultures. Some are rudimentary and primarily verbal, while others are extensive and well documented. Exam-ples of the former include medicine as practiced in the jungles and remote regions of Asia, Africa, Australia and tropical America. This form of medical practice still incorporates a sizable amount of magic or witchcraft but has captured and continues to capture our attention because it satisfies the pioneering. The end result of this effort, which is seeking clues to effective drugs, often appears to be secondary. This form of "jungle medicine" seems to be the only alternative for most American or Western researchers, due to the lack of easy access to non-English or non-European medicinal records (Herbalgram, 1990).

Medicinal Plant Research focuses on the agricultural aspects of medicinal plants that are highly utilized and will soon become extinct as they are harvested from natural environments. It is, therefore, important to propagate and cultivate the plants to ensure conservation and survival for future use (UNESCO, 1996). To support and evaluate the results of cultivation, several other research areas are explored. Determining the bio-active compounds, developing a standardisation method and determining pest and diseases on medicinal plants are some of the areas that are introduced to provide comprehensive information on the effects of propagation and cultivation on medicinal plants.

The use of plants as medicinals by the local people have been well documented as early as in 1935 by Burkill. Recent books and papers on ethnobotany and ethnopharmacology are also available but not much comprehensive. Medicinal plants were also available at that time in the markets or side walks, mostly in crude form, sold by traditional medical practitioners. Among the Navajo in the South Western United States, it is said that the bear, a highly revered animal spirit in their culture, gave them the plant *Ligusticum porteri* to use as medicine. The production of toxins, drugs and other

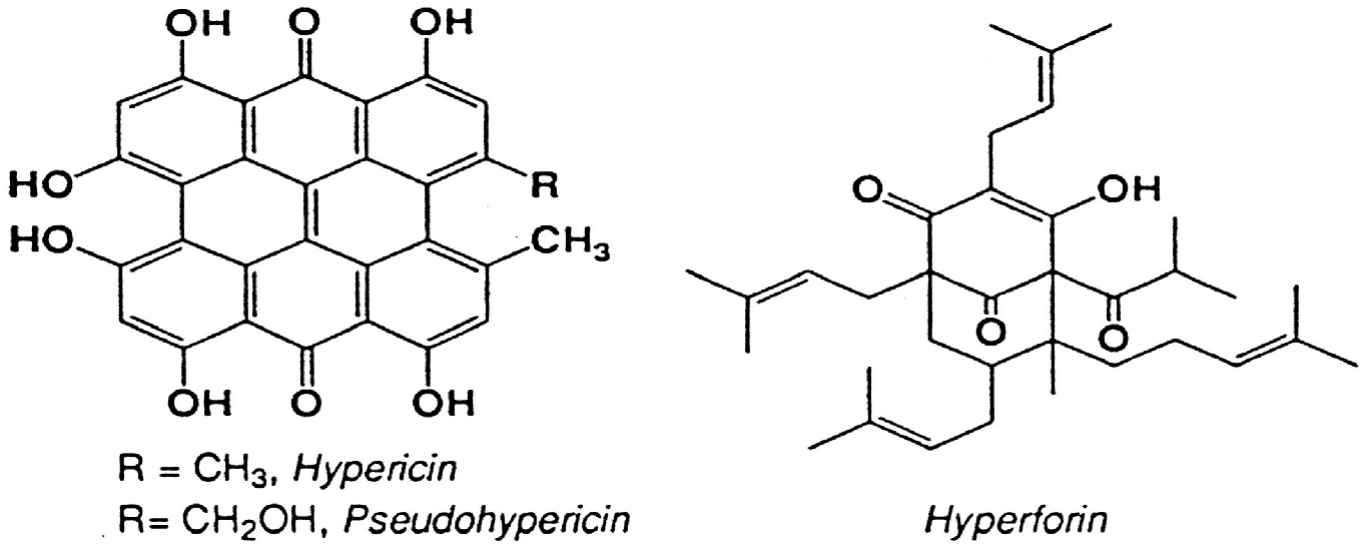

Figure 1. Hypericin, pseudohypericin and hyperforin from St. John's wort (*H. perforatum*; adapted from Bruneton, 1995).

compounds, called secondary metabolites by the chemists who study them, is considered to be an evolutionary adaptation to help plants fight off predation from insects and herbivores. The most convincing and detailed evidence for the use of medicinal plants in animals thus far comes from research on our closest living relative, the chimpanzee. Chimpanzees are susceptible to a wide range of parasite species that also infect humans. Thus far, evidence of possible use of plants as an antiparasitic adaptation come from investigations of two types of medicinal plant use, whole leaf-swallowing and bitter pith chewing (Huffman et al, 1992[b]; Rodriguez and Wrangham, 1993).

Many higher plants accumulate extractable organic substances in quantities sufficient to be economically useful as chemical feed-stocks or raw materials for various scientific, technological and commercial applications. Natural substances are employed, either directly or indirectly, by a large number of industries and natural plant products (phytochemicals; Figure 1) prominently in several of these.

Lignin, an aromatic polymer composed of dehydropolimerized structural units derived from phenylpropane and primarily responsible for the cohesion between fibres in wood tissues, is degraded and dissolved almost completely (90 - 95%) in black liquor (the aqueous solution containing the inorganic and organic reaction by-products), allowing fibre separation. In such process, wood polysaccharides, namely cellulose, hemicelluloses and less abundant polysaccharides such as pectins, are also partially degraded to low molecular weight derivatives or dissolved in the black liquor, partially keeping its polymeric nature (Sjöström, 1977; Genco et al., 1990). The concentration and composition of black liquor dissolved polysaccharides is highly dependent on wood nature and pulping conditions (Simonson, 1963; Simonson, 1965; Simonson, 1971; Söderhjelm and Hausalo, 1996).

The prenylated phloroglucinol derivative, hyperforin (Figure 2), can also be a predominant component in extracts of the flowers and leaves of St. John's wort and there is recent evidence that this phytochemical contributes to the plant's antidepressant action.

The fixed oil from the seeds of “White Todri”, *Matthiola incana*, R.Br. (Cruciferae), of Indian origin, has been studied for its component acids. The fatty acid composition was found to be myristic (2.60%), palmitic (4.73%), steric (4.37%), arachidic (2.50%), lignoceric (0.73%), oleic (32.17%), linoleic (21.70%), linolenic (10.70%), erucic (13.10%), and resin acids (7.40%). (Aziz-Ur Rahman[1] and M. Sami Khan, 2007).

One object of the present invention is achieved as follows:

Isolating oil of the aerial parts of *Rosa abyssinica* R. Br. and determination of monosaccharide composition of *Eucalyptus globulus* Wood by FTIR Spectroscopy and Isolating some new constituents in medicinal plant in Iran as such as ascalonicoside A1/A2, ascalonicoside B, rucic acid, nitrate, oxalic acid, ASCORBIC ACID, carotenoids, Monosaccharide, caryophyllene oxide and Gamma-muurolene.

MATERIALS AND METHODS

The oil was analyzed using a HP 5890 gas chromatograph equipped with a FID, an Ultra-2 capillary column (25 m x 0.20 mm; 0.25 (mu) m film thickness) and hydrogen (1.0 mL/ min) as carrier gas. GC oven initial temperature was 60°C and programmed to 240°C at 3°C/min, then kept constant at 240°C for 10 min. Injector temperature was 250°C and detector temperature was 280°C. The pure oil (0.03 (mu)L) was injected in split mode (split ratio 100:1).

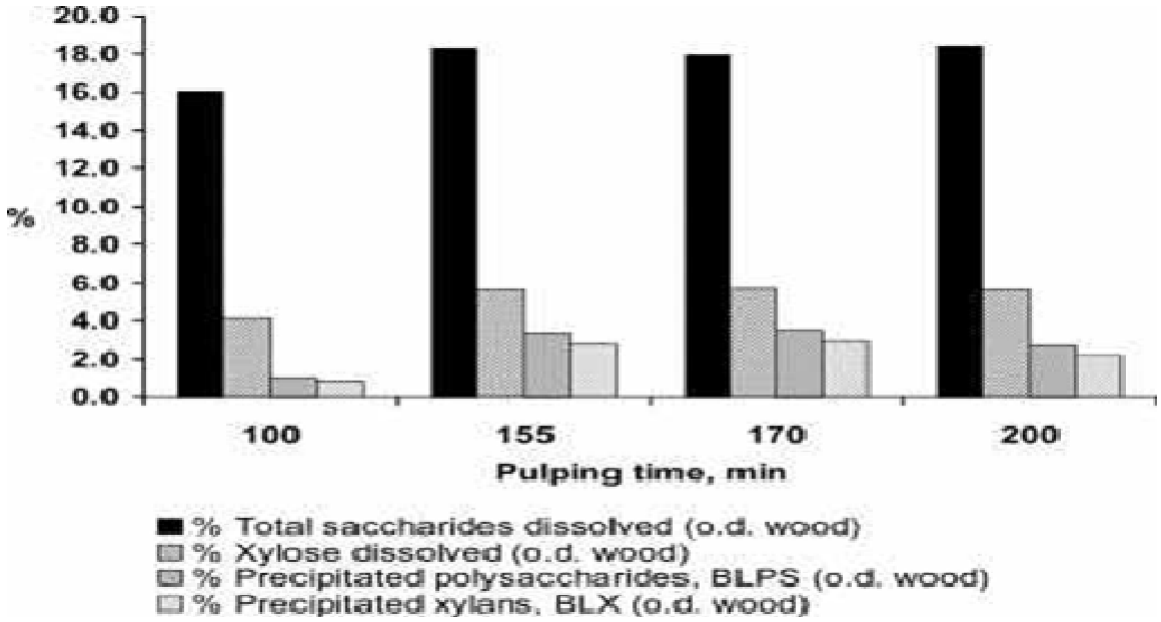

Figure 2. The balance of total dissolved saccharides (determined by difference from the wood and pulps anhydro monosaccharide composition) and black liquor precipitated polysaccharides, BLPS and BLX (xylan content is estimated as anhydroxylose content of wood, pulps and black liquor precipitated polysaccharides).

Relative quantification was calculated based on FID response in an HP 3396A integrator.

GC/MS was performed in a Hewlett -Packard 5973N system. An HP-5 MS capillary column (30 mx 0.25 mm, 0.25 pm film thickness) was used with helium (1.0 mL/min) as carrier gas. GC oven initial temperature was 60°C and programmed to 240°C at a rate of 3°C/min, then kept constant at 240°C for 10 min. The injector temperature was at 250°C. MS were taken at 70 eV. The identification of the compounds was based on mass spectra (compared with Wiley 6th edition mass spectral library), retention indices relative to n-alkanes (3) and, whenever available, co-injection with authentic samples. The content of Caryophyllene oxide and gamma-muurolene was determined by Adams method 1995.

Determination of Monosaccharide Composition of *E. globulus* Wood by FTIR Spectroscopy. The content of ascalonicoside A1/A2 (5a/5b) and ascalonicoside B were determined by (Gabriella Corea et al., 2005). The content of Phenylpropanoid glycosides determined by Kanamor method (1995). The content of Monosaccharide composition of *E. globulus* wood determined by FTIR Spectroscopy.

Carbohydrate analysis

The *E. globulus* wood (previously ground and sieved to 40 - 60 mesh), BLPS, PX and corresponding pulps were submitted to Saeman hydrolysis (treatment with H_2SO_4 72% for 3 h at 25°C, followed by hydrolysis with 1 M H_2SO_4 during 2.5 h at 100°C). The monosaccharide composition was determined as alditol acetate derivatives by gas chromatography (Selvendran et al., 1979).

Isolation of pulps xylans (PX)

About 1.5 - 2 g of pulp were dispersed in water with vigorous mixing for 1 h. The pulp was filtered off and introduced into a three neck flask. Then, 50 ml of 10% aqueous KOH solution containing 0.007 g of $NaBH_4$ was added and left to extract for 2 h at 20°C. The reaction flask was continuously purged with gaseous N_2. At the end of the extraction, the alkaline solution containing the xylan was separated from the pulp by filtration and the pulp was washed with 30 ml of KOH 10% solution and 80 ml of water. The xylan was precipitated, under agitation, by acidification with glacial acetic acid until pH 5 – 6, followed by the addition of ethanol to a total volume of 1000 ml. The solution containing the precipitated xylan was kept at 5°C for 2 days and, then, the mixture was centrifuged (after the aspiration of the major part of the clear solution) at 10,000 rpm for 20 min at 4°C. Finally, the xylan was washed with absolute methanol (three times 150 ml) and dried under vacuum and phosphorus pentoxide. The extraction yields were of the order of 60 – 80% (w/w).

RESULTS

FTIR spectroscopy was used to estimate monosaccharide content in *E. globulus* wood, using the natural variability of the species for calibration and validation of the method. A total of 38 samples from 9 year old trees from provenance trials was used. The composition ranged for glucose from 43.2% to 59.5%, for xylose from 9.4 to 17.8%, for galactose from 0.5 to 5.4%, for mannose from 0 to 2.8% and for rhamnose from 0.3 to 0.8%, based on extractive-free dry wood. The multivariate analysis gave in general, better results with increased R^2, lower SEC and SEP errors, although the univariate method also gave good fits with high coefficient of determination (R^2) based on remaining data once outliers were removed. FTIR techniques may be used in large scale breeding programmes to measure wood monosaccharide composition with considerably less effort and in shorter time than wet-lab methods, once a reliable calibration has been made for the species.

Table 1. Isolating some new constituents in medicinal plant in Iran.

Plant name	Plant Part	Components
Rosa abyssinica	Aerial parts with flowers	Caryophyllene oxide.
Rosa abyssinica	Aerial parts with flowers	Gamma-muurolene
Eucalyptus globulus	Wood	Monosaccharide
Chenopodium album L.	Wild edible plants	Ascorbic acid
Amaranthus viridis L	Wild edible plants	Carotenoids
Amaranthus viridis L	Wild edible plants	Oxalic acid
Salicornia europaea L	Wild edible plants	Nitrate
Sisymbrium irio L	Wild edible plants	Erucic acid
M. chamomilla L	Aerial parts	Phenylpropanoid glycosides
Allium ascalonicum	Red bulbs	Ascalonicoside A1/A2
Allium ascalonicum	Red bulb	Ascalonicoside B

Thirty-five components were identified representing 93.3% of the total oil. They are listed in Table (1) in order of their elution from a HP-5 MS capillary column. The oil was found to be rich in sesquiterpene hydrocarbons and oxygenated sesquiterpenes, which represented 66.6% of the oil composition. The major components were caryophyllene oxide (26.6%) and gamma-muurolene (13.0%). The total amount of monoterpenes identified was low (2.0%). The only monoterpenes identified were the monoterpene alcohols linalool (1.6%) and alpha-terpineol (0.4%).

Nutritional (ascorbic acid, dehydroascorbic acid and carotenes); antinutritional and toxic components (oxalic acid, nitrate and erucic acid) were determined in sixteen popular species of wild edible plants which were collected for human consumption in South East Spain. Ascorbic+dehydroascorbic acids contents were very high in several species, especially in Chenopodium album L. (155 mg/100 g). Carotenoid content ranged from 4.2 mg/100 g (Stellaria media Villars) to 15.4 mg/100 g (*Amaranthus viridis* L.). A range of values was found for oxalic acid from absence to 1100 mg/100 g of plant material. Nitrate contents ranged from 47 mg/100 g (*Salicornia europaea* L.) to 597 mg/100 g (*A. viridis* L.). Low amounts of erucic acid were found in the Cruciferae family (*Sisymbrium irio* L. 1.73%; *Cardaria draba* L. 1.23%) and *Plantago major* L. 3.45%.

The content of glycoside (1) was the highest among the three glycosides during the flowering time. (1) and (2) were concentrated at about 2.0% and 0.5% in the heads harvested from the end of May to the middle of June respectively. Then the content of (1) decreased rapidly but (2) decreased gradually. These two kinds of glycosides were more in the ligulate flowers than tubular flowers, but the dry weight ratio of the ligulate flowers to the tubular flowers in a head was 1/6 – 1/10. Therefore, the decreases of (1) and (2) did not seem to be attributable to the decrease of the ligulate flowers. The content of (3) became higher in an early time.

High concentrations of ascalonicoside A1/A2 (5a/5b) and ascalonicoside B (6), previously isolated from *Allium ascalonicum* Hort., were also found. This is the first report of furostanol saponins in this *Allium cepa* variety. The chemical structures of the new compounds were established through a combination of extensive nuclear magnetic resonance, mass spectrometry and chemical analyses. High concentrations of quercetin, quercetin 4^{I}-glucoside, taxifolin, taxifolin 7-glucoside and phenylalanine were also isolated

DISCUSSION

Thirty-five components were identified in the oil, representing 93.3%. The main components were gamma-muurolene (13.0%) and caryophyllene oxide (26.6%). In the cultivation of the medicinal plant, *Matricaria chamomilla* L., we investigated seasonal variation in the yield of the head (capitula) and the content of two phenylpropanoid glycosides; (1) *cis*- and (2) *trans*- 2--D-glucophyranosyloxy-4-methoxy cinnamic acid and one flavonoid glycoside; (3) cosmosiin (apigenin-7-*o*--D-glucophyranoside) in the heads harvested during the flowering time from May to July in 1993.

Then the content of (1) decreased rapidly but (2) decreased gradually. These two kinds of glycosides were more in the ligulate flowers than tubular flowers, but the dry weight ratio of the ligulate flowers to the tubular flowers in a head was $^{1}/_{6} - {}^{1}/_{10}$.

Complex carbohydrates composed of many 6- carbon sugar subunits (monomers) joined together by dehydration synthesis. Depending on the polysaccharide, the sugar subunits may consist of complex chains of glucose molecules or other 6-carbon monosaccharides. In this study we isolated monosaccharides from the wood *E. globules.*

Polysaccharides dissolved in black liquors from *E. globulus* kraft pulping were isolated by acid precipitation, analysed for monosaccharide composition and structurally characterized by methylation analysis and NMR

spectroscopy. The precipitated oligo- and polysaccharides, representing 20% of the dissolved and/or degraded wood polysaccharides are essentially composed of xylan and amylopectin. The major part of the 4-*O*-methylglucuronic residues in the xylan from black liquor were degraded to hexenuronic (4-deoxy- - l-*threo*-hex -4-enopyranosyluronic acid) units, which was not found to be so pronounced in the case for xylan in the corresponding kraft pulps.

Wood kraft delignification is generally divided into three distinct consecutive kinetic phases (Sjöström, 1993). It is worth noting that uronic acids (present in our samples, as will be seen later) were not quantified and, thus, the carbohydrate content of BLPS should be even higher than 80 - 90%, suggesting very low lignin contamination. BLPS represent about 20% of the total saccharides removed from wood in the bulk and residual phases of delignification (Figure 2) . Factors proposed affecting production of Constituents in medical plant:

The effect of different environmental factors in determining medicinal phytochemical levels in St. John's wort will be evaluated using the nutrient solution/quartz culture systems previously developed in a controlled environment growth room facility (Briskin et al., 2000). The influence of mineral nutrients will be examined by varying the levels of specific nutrients in the nutrient solution provided to the plants. While previously we have only examined the effects of nitrogen, b. we will now examine how combinations of major macronutrients and micronutrients affect medicinal phytochemical production levels. For these studies c. we will utilize a new HPLC assay whereby it is possible to determine not only naphthodianthrones but also hyperforin and active proanthocyanidins (Gray et al., 1992).

Impact to Secondary Metabolism is a major concern in Essential oil and Medicinal compound production as well. Plant productivity and its secondary metabolites can be increased by changing plant nutrition. Developing hydroponic nutrition systems affects secondary metabolites. Predictive model of secondary metabolism allows production of specific medicinal compounds, such as anticancer compounds. The methods yield a liquid and oily antioxidant extract that is readily mixed with a liquid product such as soybean oil for addition to animal feeds and human food. Foliar application of several plant-derived chemicals, such as salicylic acid and oxalic acid, can induce these defence mechanisms. The effect of acetylsalicylic acid and oxalic acid on the aphid *Myzus persicae* Sulzer. (Homoptera: Aphididae) and its parasitoid *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae) was investigated

For the plant physiologist, work on medicinal plants opens up a wide range of research possibilities and plant physiological studies would have a major role to play in this burgeoning field. The use of molecular approaches and biotechnology could also have wide application and promise especially with regard to such topics as the modification of phytomedicinal chemical pathways. Production of medicinal chemicals in plants may be modified by over-expression, antisense expression, or cosupression of biosynthetic genes. Transgenic genes could also be introduced to modify existing pathways. At present, a major concern with the use of phytomedicines regards the maintenance of consistent medicinal quality in botanical medicines (Matthews et al., 1999).

Conclusion

The Iranian people have been using their medicinals for several thousand of years. Over this long period, they have accumulated a sizeable pharmacopeia based on actual human trials, and have faithfully recorded their experience and knowledge of these medicines for posterity. Bioprospecting and the development of medicinal plants and natural products is something that the nation will have to address seriously. Isolation of active compounds from medicinal plants is not a new field any more and novel compounds are continuously discovered from medicinal plants. Investigation into the chemical composition of medicinal plants and especially secondary metabolites is a dynamic research field world wide. This is however a very important part of medicinal plant research as this provides information to compile and expand on the standardisation section of the programme.

ACKNOWLEDGEMENT

We acknowledge the financial support by the Faculty of Desertification of Semnan University.

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