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Contents

Editorial

As per World Health Organisation (WHO) study over 78% of total healthcare expenditure is out of pocket; out of it 72% is spent on medicines. Most of the Indian population is an either middle or poor class strata that's why the Union Health Minister recently issued guide lines to Pharma Industry on use of Brand name instead of generic name and for this reason since 2008 Jan Aushadhi stores have been opened nationwide. Still the fact remains that these are not the absolute solutions in India and many other parts of the World. Ayurveda or the herbal treatment is the only solution.

Scientific developments are touching new heights stem cell products as a treatment option for the medical trinity to treat various un-met needs, Preclinical diseases models are being developed to test the ability of these cells to protect the risk cells and replace cells lost to injury or disease. Human mesenchymal stem cells isolated from various post natal and adult human tissue will become an attractive tool for their potential in Cell therapy and will hit the market in near future. Nanotechnology, Cyber knife technology & Nuclear medicine etc. are many developments which have taken place today but all are expensive treatments, many are just for temporary relief from the pain and many have major side effects hence regulatory processes are strict by WHO for Therapeutic & Toxicity Tests.

Masses trust Herbal medicines that's why Liv.52 is one of the most sold Ayurvedic brand for liver care in the world. Therefore indigenous system of Ayurveda is the only answer. It is our patriotic duty to promote indigenous systems through R&D which is to be undertaken by young scientists to take up this task. This will generate employability, and consumption which will lead change in traditional cultivation of just grains which we grow more than its demand. This will help suffering farmers and give better paying crop opportunity to cultivate herbs which will compliment manufacturing of affordable herbal medicines for our indigenous health care system. Scientifically well documented Indian herbs will increase our foreign exchange, name, fame and bring prosperity to our country.

I conclude by thanking all those who have contributed for their monetary help in bringing out this issue and to all the Board Members of UJPAH, contributors of research papers, participants, eminent scientists and not the least Dr. I. P. Saxena for bring out this journal and in time organizing this seminar with the contribution of Dr. I. P. Pandey & Mr. Sanjay Jain.

My good wishes to all and a very happy new year.

Dr. S. Farooq
Chief Editor

Sustainable Development of Medicinal and Aromatic Plant Sector

Sustainable Development (SD) of Medicinal and Aromatic Plant (MAP) Sector usually refers to its long term development. Earning profits for some time out of sale of collected and cultivated raw materials and their processed products does not imply that its development has been sustainable. SD has been widely and variously defined but consensus as to its general implication that SD requires a non declining level of well being for future people. The concept of SD developed from earlier understanding related to impact of human activities on prosperity, health and environment.

Ethics, Ecology and Economics issues are basically involved in the complex concept of SD. There is need to give importance to the integration of the above three E's to achieve Sustainable Economy. Ethical principles emphasize on our right to respect and conserve natural resources and on its fair distribution. Environment (a sub-set of Ecology) relates to life support systems, atmosphere, land and water. These natural resources have to be maintained and not allowed for degradation during economic development of any sector. Economic perspective addresses problems like distribution of wealth and non-monetized values related to environmental conservation. Ecological economics is now a new trans-desciplinary way to look at the sustainability of the system / sector.

Economic aspects related to MAP sector can be developed through various strategies viz. improved techniques of cultivation, harvesting, processing technologies, storage and marketing. Selection of rare and elite plants for cultivation will fetch high cost and value addition at the source of harvesting to raw materials will increase the economic value.

Besides, two other important strategies are, empowering farmers with (1) Intellectual Property Rights (IPR); this would protect traditional knowledge on medicinal plant through patents and (2) Benefit sharing which means sharing of profit earned by manufacturers and the farmers who supply the herbs. Farmers who grow herbs of therapeutic value should be given 2 to 3% of profit earned by manufacturers who utilise their herbs. A policy that does not obstruct advancement of knowledge and provides for valid sustainable uses and intellectual property protection with just benefit sharing is what we need. These steps, if taken would go a long way to encourage farmers to adopt fast lane for cultivation of MAPs.

The above strategies alone would not in any way increase economics of the sector unless they are interlinked with ethics and ecology. If all the above factors are as integral part of each other, sustainability of the Sector can be achieved.

We owe these guidelines to current and future generations to make that start now.

I.P. Saxena
Editor

Isolation, Biotransformation and Evaluation of Antibacterial Activity of Embelin from *Embelia ribes*

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Abstract - Embelin is a major chemical constituent isolated from Embelia ribes, commonly known as "vidang" which belongs to the family Myrsinaceae and is generally seen in areas of eastern India like Meghalya and Assam. Embelia ribes is a constituent of various hepatoprotective, anthelmintic and anti-fertility formulations and preparations. In the current study embelin was extracted from the fruits of Embelia ribes using soxhlet extractor and silica gel column chromatography, biotransformation of the isolated embelin was then carried out using different microorganisms namely Penicillium chrysogenum, Aspergillus niger, Pseudomonas fragi, Acetobacter spp., Candida spp. and Lipomyces lipofer. Lipomyces lipofer successfully biotransformed the embelin into a new related compound with molecular mass less than that of embelin. Furthermore, the antibacterial activity of pure embelin against different pathogenic bacteria was evaluated using disk diffusion method. Embelin has shown significant activity against Bacillus subtilis and Streptococcus mitis, weak activity against Staphylococcus aureus and it has not shown any activity against Escherichia coli. The new compound obtained by the biotransformation of embelin might have increased biological activity which could provide improved results in the antimicrobial assay than that of embelin.

Keywords: Biotransformation, Embelin, Antimicrobial activity.

Introduction

Plants, since times immemorial, have been used in virtually all cultures as a source of medicine. The widespread use of herbal remedies and healthcare preparations obtained from commonly used herbs and medicinal plants has been traced to the occurrence of natural products with medicinal properties. Thousands of natural products obtained from plant sources have been used by mankind for alleviating diseases and/or their symptoms. As human beings are threatened by diseases that require innovative therapies, all over the world, researchers/Scientists are involved in exploring the sources of new compounds, bioactive molecules for drug discovery. These new chemical entities can be natural or synthetic. Naturally occurring compounds may also be structurally modified by chemical and/or biotransformations (Adams et al., 2003).

Biotransformation has emerged as a new technology in the field of natural products. It is a method of maximizing diversity from a single chemical entity by using other biological systems in the form of whole cells or isolated enzymes to modify the molecule. The ability of enzymes or microbial catalysts to perform difficult chemical reactions such as hydroxylation of non-activated carbons on structurally complex molecules without the need of protection and deprotection steps of reactive functional groups was recognized. There are many reasons worth trying for exploiting biotransformation in the chemistry of natural product.

Biotransformation encompasses the use of living organisms such as fungi, bacteria, microalgae, yeast and plant cell as biocatalysts. The substrate specificity of many of these microorganisms for bioconversion is often quite low, enabling a single organism to biotransform a range of related compounds with similar efficiencies. Moreover, enzymes that are produced by microorganisms are well known for practically every type of chemical reaction. The chemical reactions occurring in biotransformations include oxidation, reduction, hydrolysis, condensation, isomerization and formation of new C-C bonds (Satyanarayana, 2005).

Embelin is the major chemical constituent isolated from Embelia ribes, commonly known as "vidang". Vidang is used in many ayurvedic and herbal formulations; dried fruits are used as anthelmintic, astringent and alterative tonic for ascariasis, scorpion-sting and snake-bite (Chitra et al., 2003) and aqueous extract of fruits possess antibacterial and antifertility activities (Nayar et al., 1999). Seeds were found to possess antibiotic and anti-tubercular properties (Chitra et al., 2003).The present study was aimed to isolate the Embelin compound from the fruits of Embelia ribes (Myrsinaceae) and this

compound was subjected for biotransformation using six microorganisms. The antimicrobial activity of pure embelin was also studied against pathogenic bacteria.

Materials And Methods

Standard of pure embelin and dry fruits of vidang were obtained from the Department of Natural Products, National Institute of Pharmaceutical Education and Research (NIPER) Mohali, Punjab. All the microbial strains i.e., Aspergillus niger, Penicillium chrysogenum, Candida spp., Acetobacter spp., Pseudomonas fragi, Staphylococcus aureus, Escherichia coli, Streptococcus mitis, Bacillus subtilis were obtained from college depository, Dolphin College of Life Sciences, Chunni Kalan, Punjab. Lipomyces lipofer was procured from Institute of Microbial Technology, Chandigarh, India.

Isolation and characterization of embelin

Coarsely powdered fruits of E. ribes (500 g) were extracted with methanol by hot soxhlet extraction for 8 hours. The crude extract obtained was concentrated on a rotary evaporator to yield brownish red slurry (Kumara Swamy et al., 2007). This slurry was further processed to isolate embelin in pure form (Fig.1).

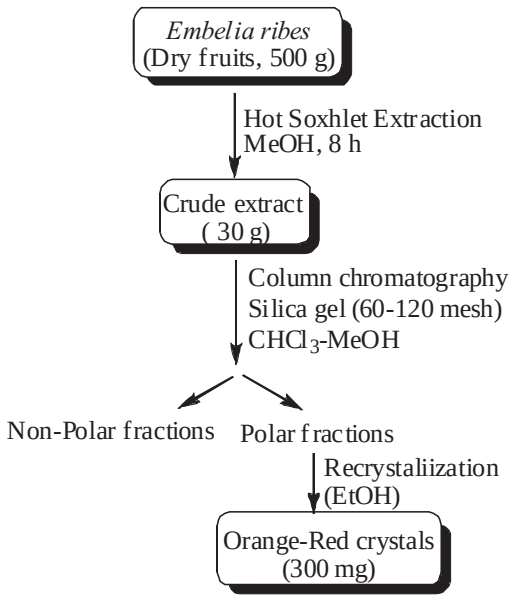


Fig.1: Protocol for isolation of embelin from E. ribes.

The purity of the compound was checked by comparison with pure embelin compound over pre-coated silica gel TLC plate. The Mass and NMR spectroscopy was performed for the compound extracted and the data of Mass and NMR was compared with that of pure embelin.

Biotransformation of embelin

The compound isolated in pure form was subjected for biotransformation by six different micro-organisms one by one. The specified media's recommended for proper growth of micro-organisms were prepared, autoclaved and inoculated with test compound (10 mg dissolved in 1 ml of DMSO was added to 50 ml of media) (Miyazawa et al., 2005).

The inoculated media were incubated for one week on rotary shaker incubator at 28 °C for fungi and yeast and at 37 °C for bacteria. Positive and negative controls were run along with the test sample. Positive control, containing media (50 ml) inoculated with micro-organism, was run to check for any possible contamination. Negative control, containing media (50 ml) and the test compound (10 mg) dissolved in 1 ml of DMSO (as in test sample), was run to ensure that no extra compound was generated in the mixture as a result of interaction of media components with embelin and/or DMSO. At the end of the fermentation period the media were filtered. Both the filtrate and biomass were exhaustively extracted with ethyl acetate (20 ml, three times). The combined ethyl acetate extracts were dried over sodium sulphate and concentrated under vacuum at 40°C. The generation of any new compound was monitored by thin layer chromatography over silica gel plates using propanol : butanol : ammonia (7:2:1) as solvent system. Also, to confirm the formation of any new products, the microbial broth was extracted with ethyl acetate and the ethyl acetate layer was dried. The residue left behind was dissolved in methanol and subjected to mass spectrometry in atmospheric pressure chemical ionization (APCI) mode. The emergence of any new molecular ion peak (apart from the peak of embelin) could be correlated to formation of biotransformation products.

Anti-bacterial activity of pure embelin

Anti-bacterial activity of embelin was studied by disk diffusion method using four different species of bacteria namely Staphylococcus aureus, Escherichia coli, Bacillus subtilis and Streptococcus mitis. Pure embelin was dissolved in Dimethylformamide (DMF) and concentrations of 25, 50 and 100 µg/disk were used for the study (Chitra et al., 2003).

Petriplates were inoculated with the above mentioned bacterial species. Now the sterilized disks with different concentration i.e., 25, 50 and 100 µg/disk of embelin were placed on the culture plates. One disk dipped in only DMF was placed as control to check the

antimicrobial activity of DMF. Then plates were incubated at 37 °C for two days.

Results And Discussion

Isolation and characterization of embelin

Crude methanolic extract of E. ribes was chromatographed over silica gel using chloroform-methanol as eluent. The polar fractions eluted with 50% methanol in chloroform were checked for the presence of embelin by thin layer chromatography. Fractions showing the spot for embelin were eluted and concentrated to yield 300 mg of a dark orange crystalline compound.

The isolated compound was characterized by Mass and NMR spectroscopy. It was found that the compound showed a molecular ion peak at m/z 294 confirming the mass of the embelin (Fig.2). The proton NMR spectrum of embelin showed a one proton signal at ? 6.00 ppm for the proton attached to the benzoquinone ring. The hydroxyl group attached to ring was observed at 7.68 ppm. A triplet for three protons of terminal methyl group of long alkyl chain was present at 0.86 ppm. Other signals for methylene residues of alkyl chain were present in the region 1.25 to 1.29 ppm (Fig.3 & 4).

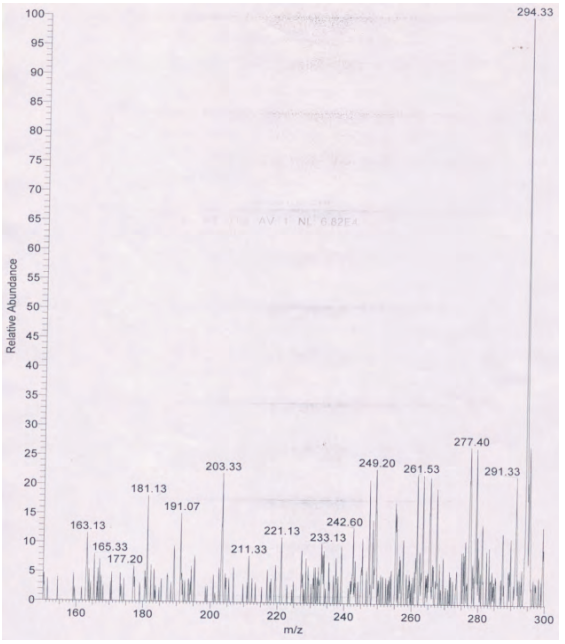


Fig.2: Mass spectrum of embelin.

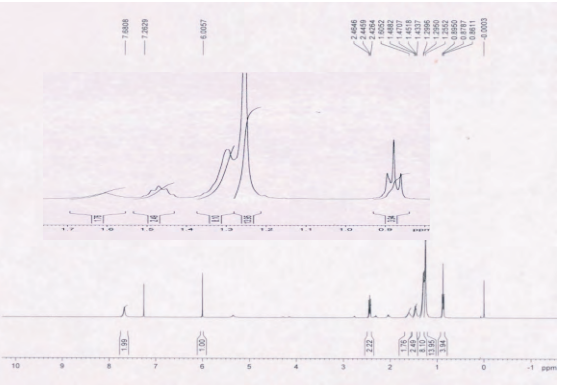


Fig.3: 1H NMR spectrum of embelin.

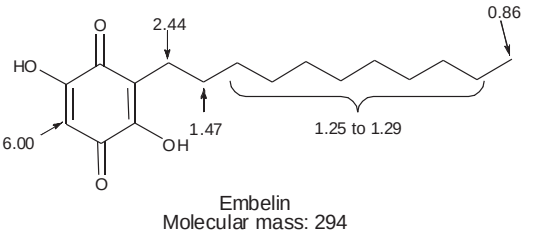


Fig.4: Structure of embelin.

Bio-transformation of Embelin

Bio-transformation of embelin was carried out using six different strains of microrganisms. The course of transformation was monitored by TLC after a period of one week. Embelin biotransformation was not observed in case of Penicillium chrysogenum, Aspergillus niger, Pseudomonas fragi, Acetobacter spp. and Candida spp. On TLC plates we observed the spot for embelin at same Rf (0.34) as that of standard embelin which suggested that biotranformation of embelin has not occured. In case of Lipomyces lipofer, after an incubation period of 14 days, no spot for embelin could be detected on TLC rather a very faint polar spot appeared that did not move from the point of application.

The concentrated ethyl acetate layer of the microbial broth of L. lipofer was then subjected to mass spectrometry. The molecular ion peak of embelin that appears at m/z 294 was found to be absent in this mass spectrum. Rather an intense molecular ion peak at m/z 238 [M-2]+ was observed. This molecular mass could be attributed to an oxygenated compound with same structural skeleton as that of embelin, wherein the C11 alkyl chain of embelin was cleaved to C5 chain oxygenated to a carboxylic acid group at the terminal carbon (Fig.5). The mass spectroscopic analysis suggested that the embelin compound was bio transformed by L. lipofer.

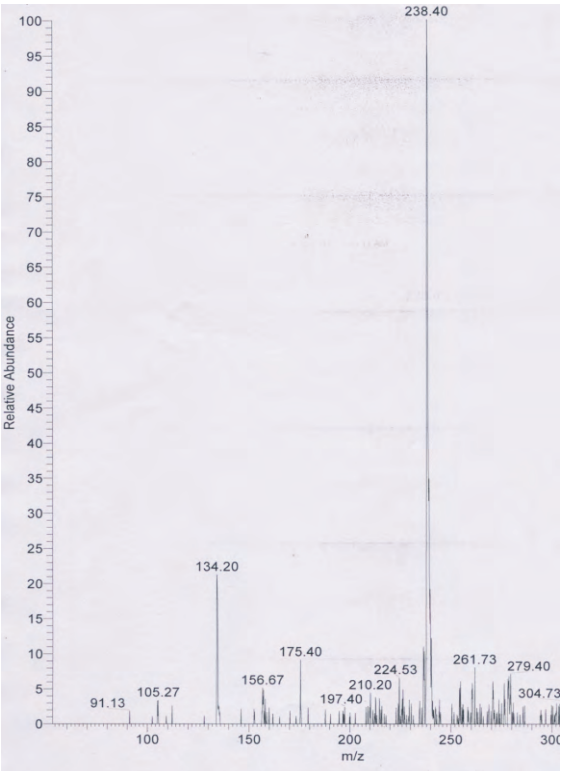
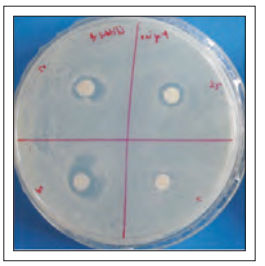
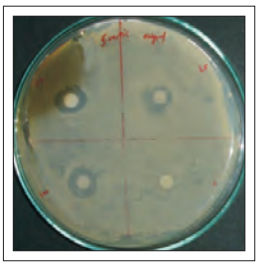


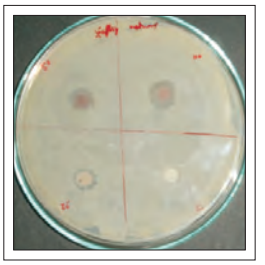
Fig.5: Mass spectrum of transformation product of embelin.



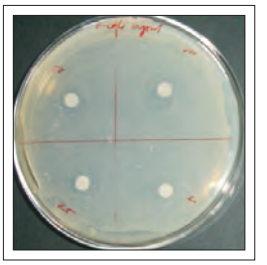
Bacillus subtilis



Streptococcus mitis



Staphylococcus aureus



Escherichia coli

Antibacterial Activity

Pure Embelin was found to be active against Streptococcus mitis and Bacillus subtilis. It showed a weak antibacterial activity against Staphylococcus aureus and no antibacterial activity against Escherichia coli (Table-1).

Table-1 Antibacterial activity of Embelin

S. No.	Micro-organism	Gram + Or Gram -	Zone of inhibition(mm)		
			25 µg	50 µg	100 µg
1	Bacillus subtilis	G +	9	10	15
2	Streptoco--ccus mitis	G +	9	13	13
3	Staphyloc--occus aureus	G +	18	20	28
4	Escherichia coli	G -	-	-	-

Embelin is a banzoquinone isolated from various plant sources like Embelia ribes, Myrsine Africana and Lysimachia punctata. Fruits of E. Ribes being a rich source of embelin (2.5 to 3%) were subjected to hot soxhlet extraction with methanol and the compound of interest was isolated by silica gel column chromatography. The authenticity of the isolated compound was checked by thin layer chromatography over silica gel by running a co-TLC with standard compound, 1H NMR and mass spectroscopy.

In the present study Lipomyces lipofer was the only micro-organism that could transform embelin into another product. Similarly, the Lipomyces lipofe has been reported for the biotransformation of maesanin, a benzoquinone structurally related to embelin (Abourashed et al., 2000). Similarly, Biotransformation of 2,2-dimethyl-1,3-propanediol into 3-hydroxypivalic acid and11-?-Hydroxy progesterone was observed in Acetobacter spp. (Molinari et al., 2003) and Aspergillus niger (Miyazawa et al., 1995) respectively. In the present study the biotransformation product of embelin was obtained in very low yields and was partially characterized by mass spectrometry. The molecular ion peak present at m/z 238 suggested that the parent compound embelin (m/z 294) has been biotransformed by the microbe and a new compound in which the aliphatic carbon chain was partially cleaved had emerged as a product.

The antibacterial assay of embelin suggests that it is not a very efficient antibacterial compound but could give

rise to more potent bioactive molecules by biotransformation which is not extensively implemented in the case of embelin. Lipomyces lipofer is suggested to use as a model for biotransformation assay for embelin because it is able to bio transformed embelin in to new modified compound. The new compound obtained by the biotransformation of embelin might have increased biological activity which could provide improved results in the antimicrobial assay than that of embelin. Further study is undertaking.

Acknowledgements

We are thankful to Natural product Department of NIPER, Mohali to provide with the dried fruit of E. lipfer and pure compound of embelin and Dolphin (PG) College of Life Sciences, Chunni-Kalan, for providing infrastructure and facilities for research.

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Isolation and Characterization of New Flavone from flowers of *Reinwardtia indica*

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Abstract - A new flavone 1 has been isolated from the alcoholic extract of the flowers of Reinwardtia indica together with known compounds, ?-sitosterol and its ? - D-glucoside. The structure of 1 was elucidated as 7-methoxy-3'-acetoxy-5'-hydroxy-6-methene-[Napthalene,decahydro-3"-hydroxy-2",6",7",10"-pentmethyl]-flavone with the help of chemical and spectral studies.

Introduction

Reinwardtia indica syn. Linum trigynum; vern. Phiunli, belongs to family Linaceae. Yellow Flax is an erect to spreading shrub, up to 1 m tall, and is commonly found in a prostrate state. Leaves are elliptic to invert lance-shaped. Midday in December or January, two dozen yellow flowers that is open. Each golden-yellow flower, 5 cm wide, lasts that one day and is described as being fugacious, meaning that the corolla withers, and falls off easily. A yellow dye made from the flowers is used for dyeing clothes and making paints. Flowers were also analysed for their essential oil composition useful in different cosmetic industries. Yellow Flax is found from Pakistan to SW China, at altitudes up to 1800 m. It is a common wildflower of north-Indian hill-stations [Gaur, 1999; Lemmens et al, 1991; Wealth of India, 1988].

The present paper deals with the isolation and structure elucidation of a new flavone 1 from the alcoholic extract of flowers of Reinwardtia indica together with two known compounds

Material and Methods

General Experimental procedure

Melting points were recorded on a Perfit melting point apparatus. UV spectra were measured on a Perkin-Elmer Lambda-25 spectrophotometer in methanol. IR spectra on a Perkin-Elmer Spectrum RX1 FT-IR spectrometer (KBr discs). NMR spectra were obtained on Bruker Avance 300 and 500 spectrometers (300 MHz for 1H and 125 MHz for 13C, $CDCl_3$ as solvent, (TMS as internal standard). MS were recorded on Atmospheric Pressure Ionisation Mass Spectrometer (APIMS). Column chromatography was performed on silica gel (Merck 60-120 mesh, 15 × 100 cm).

Plant material

Stem bark of Reinwardtia indica were collected from Mangroli Nandprayag, Garhwal, Uttarakhand, India and identified from the Plant Identification Laboratory, Department of Botany, H.N.B. Garhwal University Srinagar. A voucher specimen was deposited in the Department for future records.

Extraction and isolation

The air dried flowers of Reinwardtia indica (2 kg) were powdered and extracted exhaustively with 95% ethanol (3 times) to yield a yellow extract, which was concentrated under reduced pressure and defatted with n-hexane. The extract (200 g) was pre-adsorbed with silica gel and applied on the top of a column prepared by silica gel (600 g) in $CHCl_3$. The elution was first started with $CHCl_3$ and then $CHCl_3$ with increasing amounts of MeOH. Elution with $CHCl_3$:MeOH (83:17) afforded compound 1, whereas 97:3 and 85:15 furnished ?-sitosterol and its ? - D-glucoside .

Results and Discussion

Compound 1 was obtained as white amorphous powder from methanol. It gave green colour with $FeCl_3$ and positive Shinoda test (Mg/HCl) characteristic for flavonoids. It furnishes a molecular ion peak observed at m/z 563.693 [M+H]+, which correspond the molecular formula $C_{34}H_{42}O_7$. Other fragmentation peaks at 503, 531, 248, 255, 231 and 225 in its LCMS. UV spectrum of compound showed characteristic flavones absorptions at 272 and 336nm and its IR spectra showed characteristic absorption bands at 3500 for hydroxyl and 1660 for carbonyl functions. The 1H-NMR spectrum of compound confirmed the presence of a flavones skeleton characterized by three singlets at ?7.26, 7.18 and 6.82 assigned to H-2', H-4' and H-6' respectively (Table-1)

13C-NMR spectrum showed 34 signals suggested that 34 carbons are present in the molecule. In addition the presence of doublet signal of methylene at ?2.13 and 2.15 for H-9 attached at C-6 which was further confirmed by

13C-NMR at ?29.49.One singlet at ?3.63 (3H) was accounted for the presence of methoxyl group present in the compound. The upfield doublet for H-1" at ? 1.77 showed naphthalene decahydro group attached to C-1". HMBC spectrum showed correlation between H-9 and H-1" supported the position of naphthalene decahydro group (Gopalakrishnan 2011). The downfield signals in 13C-NMR at ?174.21 (C-4) and 166.8 (C-3") indicated the position of carbonyl function. On the basis of spectral data (IR, UV, 1H, 13C, 2D-NMR, and MS) and compare with literature values. On the basis of these finding, the structure of compound was characterized as 7-methoxy-3'-acetoxy-5'-hydroxy-6-methene-[Napthalene,decahydro-3"-hydroxy-2",6",7",10"-pentmethyl]-flavone (Fig.1).

An amorphous powder (MeOH, 115mg); m.p.243-245°C; UV (nm): 272, 336; IR: 3500, 1660 cm-1; 1H and 13C NMR data: Table 4.6; LCMS: 563[M]+; 503 [M-COOCH3]; 531 [M-OCH3]; 230,255, 224 .

Table - 1 . 13C (125 MHz) and 1H NMR (300 MHz) data of compound in DMSO d6

Position C/H	$\delta_c$ppm	$\delta_H$ppmJ(Hz)	Position C/H	$\delta_c$ppm	$\delta_H$ppmJ(Hz)
2	163.63	-	1"	52.93	1.77(d,7.2)
3	113.93	6.65(s)	2"	40.11	1.73(d,7.2)
4	174.21	-	3"	76.99	3.86(dd,9.5,9.5)
5	153.65	-	4"	32.16	1.67(d,7.2)
6	121.95	7.14(s)	5"	51.22	1.79(d,7.2)
7	163.63	-	6"	33.34	-
8	124.42	-	7"	37.83	1.59(d,7.2)
9	143.69	6.53(s)	8"	26.94	-
10	130.77	-	9"	29.52	-
11	29.49	2.13,2.15	10"	29.87	-
1'	122.95	-	11"	14.47	1.26(s)
2'	128.56	7.26(s)	12"	20.50	1.82(s)
3'	132.12	-	13"	20.50	1.82(s)
4'	115.29	7.18(d,8.4)	14"	16.55	1.27(s)
5'	141.77	-	15"	18.0	1.32(s)
6'	138.20	6.82(d,8.4)	OCH_3	56.04	3.63(s)
			$COOCH_3$	16686	-
				23.21	2.21(s)

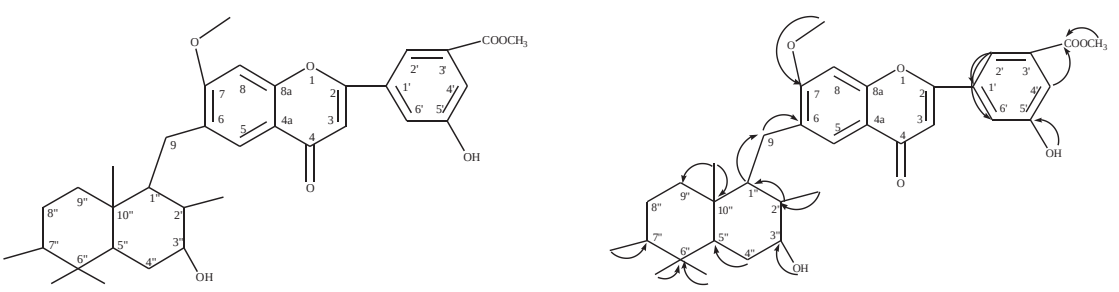
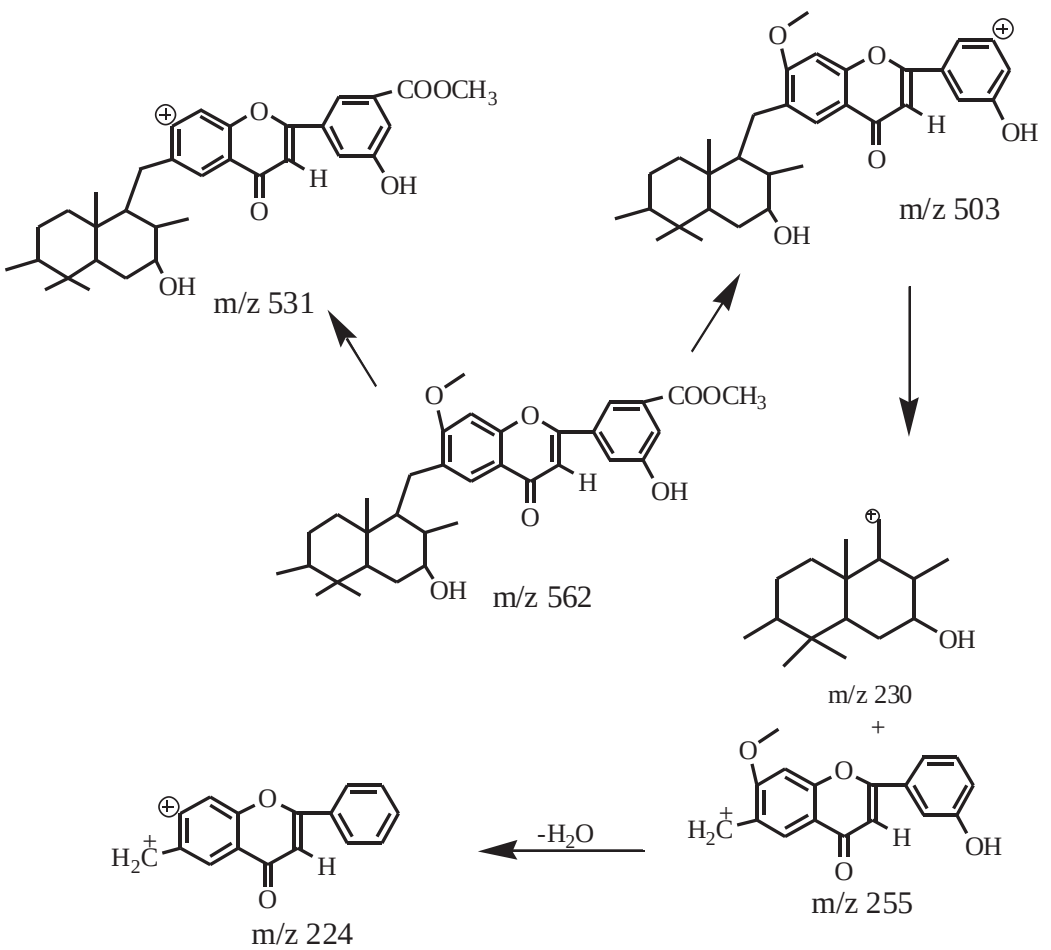


Fig.1



Proposed mass fragmentation of Compound

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A New and Safest Substitute of Allopathic Medicine for Diabetes

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Abstract - There is no permanent cure for diabetes and some times post meal blood glucose remain uncontrolled even on taking the oral drugs and insulin, therefore a newer treatment was constantly needed. After an intensive research, this problem was solved by inventing diabetes herbal oil. A precise number of drops of this oil neutralize excess of glucose present in blood stream and reduce/or control blood glucose level up to normal range (100-140 mg/dl) within two and half an hours after massaging over both the legs between knees to ankle portion. Contrary to oral drugs and insulin, diabetes herbal oil has no binding to be used daily. As soon as the excess blood glucose is neutralized, the process of neutralization stops till week to prevent low blood sugar condition (hypoglycemia) on account of entero hepatic circulation. Therefore patients are applying the oil after an interval of one week.

Now diabetic patients don't have to spend days and weeks anxiously waiting for blood glucose to come normal. Weekly usage of diabetes oil can solve the existing problem of highs and lows of blood sugar of people suffering from uncontrolled blood sugar level. The diabetes herbal oil has a big advantage that it can cover all the type of diabetes like Type-1, Type-2, gestational and juvenile diabetes.

Keywords : Diabetes herbal oil, post prandial glucose, Type-1 and Type-2 Diabetes, Gestational and Juvenile Diabetes.

Introduction

The pancreas, an organ that lies near stomach, makes a hormone called insulin to help glucose get into the cell of our bodies. High levels of insulin resistance can lead to the development of diabetes. Insulin resistance is mainly caused due to lack of physical activity but pancreas is not destroyed. In allopathy, treatment of diabetes is done by oral anti diabetic drugs and artificial insulin. Monitoring of oral drugs and insulin is still a big problem and blood glucose level in the patients always remain either higher than normal level (hyperglycemia) due to under dose or lower than normal level (hypoglycemia) due to over dose, which can lead to a series of complication in brain, kidneys, heart, eyes, nerves and major arteries.Anti diabetic oral drugs and artificial insulin are also known as Amino Derivatives because both of them contain amino functional group. Their mechanism take place by glucuronic acid pathway. In the glucuronic acid pathway of glucose utilization, first of all glucose1 from blood enter into the liver cells by insulin independent transport system and enzyme glucokinase convert glucose to glucose- 6- phosphate. Phospho glucomutase catalyses the conversion of glucose 6 phosphate to glucose -1- phosphate.Now glucose -1- phosphate and U.T.P. form uridine di phosphate glucose (UDPG) in presence of UDP-glucose pyrophosphorylase. The glucose portion of UDPG is oxidized to yield uridine diphosphate glucuronic acid (UDPGA). This UDPGA ultimately conjugates with oral drugs or artificial insulin through their -NH-group in presence of Glucurnoyl transferase to form a polar compound by the process known as Glucuronidation 2 The polar compound called, N glucuronide is more easily cleared from blood through kidneys and excreted in the urine without being hydrolyzed in the small intestine as is observed in case of bile salt during entero hepatic circulation. This is the main cause of the condition of hypoglycemia when these drugs are by chance taken in excess.

In all the human beings it is the blood glucose which is transformed into the energy with the help of natural insulin and glucagons hormones secreted by pancreas without any compulsion of doing any physical activities like walking or exercises. But in diabetic patients energy transformation is only possible when pancreas is activated by exercise or walking. If diabetic patients avoid exercise their high blood sugar forces liver to rely less on conversion of glucose to glycogen and more on conversion of glucose to saturated triglycerides(fat). This causes much load to the thyroid gland which regulates the triglyceride levels and hormonal imbalance starts in side the body. These drugs also impart obesity, patient becomes reluctant to these necessary exercises or walking and the blood glucose remains unutilized by the body and is excreted through the kidney after its complete neutralization by these drugs. Patients ultimately become susceptible to Osteoporosis, Arthritis and Blood pressure in long run.

Discussion

From the following equation

$C_6H_{12}O_6 + 6\ O_2 + 38\ ADP + 38pi$---------- $6CO_2 + 6H_2O + 38$

ATP (682 K. Cals)

It can be calculated that when 180000 mg glucose is metabolized during aerobic respiration 682,000 Calories energy is released and generally it has been observed that by walking one kilo meter a person can approximately burn his 60 calories. Hence a novel glucose/calories/exercise, equation3 can be given as follows:

60 calories = 20 mg/dl glucose = one kilo meter walking

Which means to reduce 20 mg/dl blood glucose, people suffering from diabetes should cut in 60 calories -laden carbohydrates and fat from their diet or they should burn out same calories by means of exercise or walking. For the selection of desired calorie meals they can consult new calories/blood glucose/walking table-1.

Table-1, Calories/ blood glucose/ walking

FRUIT /100 GRAM	CALORIES	GLUCOSE Mg/dl	WALKING k mt
Apple	56	17	1.0
Avocado	190	57	3.0
Banana	95	28	1.5
Chickoo	94	28	1.5
Cherries	70	20	1.0
Dates	281	84	4.0
Grapes black	45	13	1.0
Guava	66	20	1.0
Lychies	61	18	1.0
Mangoes	70	21	1.0
Papaya	32	10	0.5
Watermelon	26	08	0.5
Pine apples	46	14	1.0
Pears	51	15	1.0
Strawberries	77	23	1.0
Pomegranates	77	23	1.0
Orange	53	16	1.0
VEGETABLES			
Broccoli	25	07	0.5
Brinjal	24	07	0.5
Cabbage	45	13	1.0
Carrot	48	15	1.0
Cauli flower	30	09	0.5
Fenugreek	49	15	1.0
French beans	26	08	0.5
Lettuce	21	07	0.5
Mushroom	18	06	0.5
Onions	18	06	0.5
Peas	93		28
1.5			
Potato	97	30	1.5
Spinach	26	07	0.5
Tomato	21	06	0.5
MILK PRODUCTS			
Butter Milk	19	06	0.5
Cheese	315	100	5.0
Ghee	910	273	11
Milk buffalo	115	35	02
Milk skimmed	45	13	01

Milk (Cow)	100	30	1.5
FLOURS			
Gram flour	387	130	6.5
Wheat flour	339	113	6.0
Barley flour	352	117	6.0
Soya bean flour	446	148	7.0
Bajra flour	360	120	6.0
Oat flour	389	129	6.0
Maize flour	355	118	6.0
Ragi flour	324	108	5.0
Rice flour	325	108	5.0
Sorghum flour	339	113	6.0
Indian Dals (Lentils)			
Arhar Dal	53	18	1.0
Chana Dal	99	33	1.5
Dal Makhani	117	39	2.0
Moong Dal	211	70	3.5
Masoor	165	51	2.5
Urad	107	39	2.0
Chiken Curry	132	44	2.0
Rogan josh	500	166	8.0
Fish	96	32	1.5
Upma	70	23	1.0
Uttapam	150	50	2.5
Vegitables and Beans Soup	140	46	2.0
OTHER ITEMS			
(One tea spoon)			
Honey	64	21	1.0
White sugar	15	05	0.5
Refined oil	40	13	1.0
Butter	33	11	0.5
Extra Virgin Olive Oil	40	13	1.0
Tomato Catchup	15	05	0.5
Mayonnaise	57	20	1.0
Jam	55	18	1.0
Margarine	17	06	0.5
One Chapatti	119	40	2.0
One Slice white	60	20	1.0
One Paratha	282	74	4.0
One Egg	84	28	1.0
One Cheese Burger	300	100	5.0
One Besan Laddu	315	105	5.0
One Samosa	252	84	40
One Rasgulla	194	64	3.0
One Paneer Pakoda	376	125	6.0
One Sandesh	30	10	0.5
One till Laddu	55	20	1.0
One Kaju roll	83	27	1.0
One Aloo Tikki	101	30	1.5
One Idli	85	28	1.0

Distribution of calories in some food items/100g

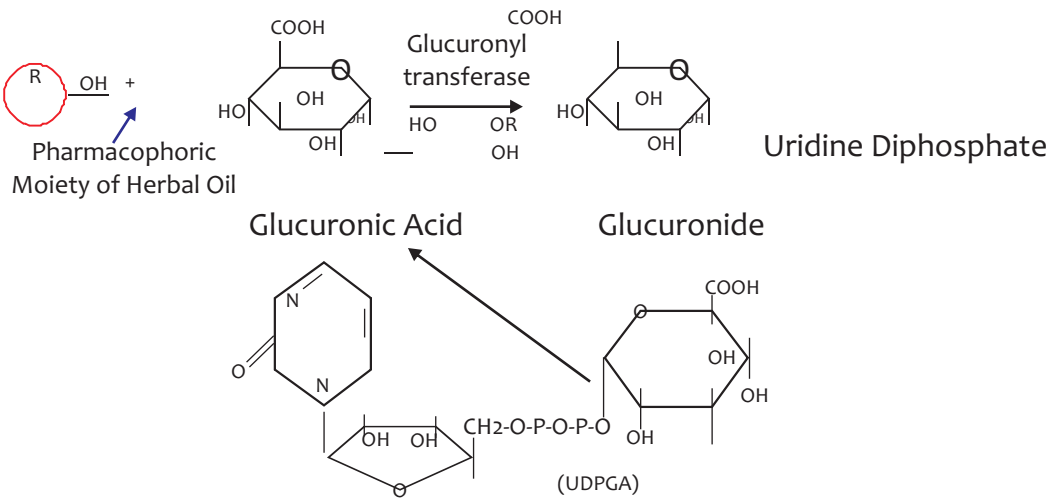
	Carbohydrates	Sugars	Fats	Proteins	Dietary Fibers
Gram flour	57	10g	6g	22g	10g
Wheat flour	72.5	0.41	1.87	13.70	12.2
Barley flour	77.7	0.8	1.2	9.9	15.6
Soya bean flour	30.16	7.33	19.96	36.49	9.3
Bajra flour	73	----	4.2	11.00	8.5
Oat flour	66.3	----	6.9	16.90	10.06
Maize flour	19.02	3.22	1.18	3.22	2.7
Sorghum flour	75	1.9	3.3	11.3	6.3
Ragi	72	----	1.4	7.3	3.6
Chana dal	27.42	4.8	2.59	8.86	7.6
Moong dal	6.60	----	1.15	23.86	16.3
Arhar dal	8.0	----	1.2	2.8	----
Masoor dal	24.7	----	2.7	10.3	20.8
Urad dal	14	----	3.0	6.0	----
Dal makhani	14	----	1.3	6.8	----
Sambhar	2.6	----	15	----	----
Yoghurt	3.0	----	4.1	3.1	----

Experimental

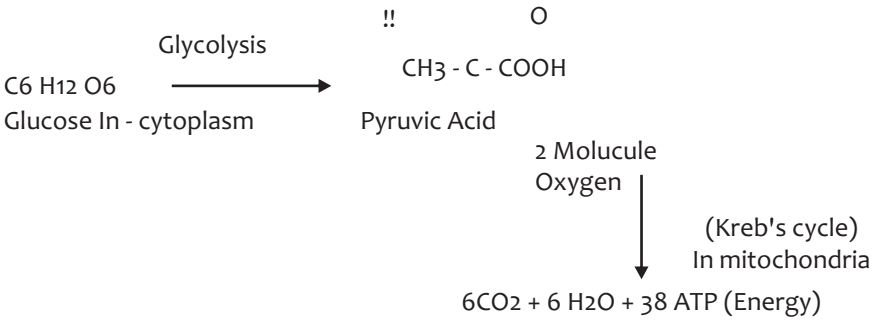
Herbal oil is a mixture of various poly- phenols which have been extracted from different medicinal plants by fractional distillation. Since they contain hydroxyl groups as pharmacophoric moity (fig.1), they form O-Glucuronide with UDPGA and in case of overdoses the O-glucuronide is accumulated in the Gall-bladder and released into the small intestine and can be hydrolyzed by bacterial enzyme to give back UDPGA and there is no risk of hypoglycemia due to entero hepatic circulation.

Thus the herbal oil may be used weekly because as soon as the excess blood glucose is neutralized on first application, the next process of entero hepatic circulation automatically starts and lasts for approximately six days, to protect from hypoglycemia.

Fig.1 Mechanism of Polyphonols in the formation of Glucuronide



Mechanism of Glycolysis in presence of natural insulin to produce energy



Toxicity Report

Acute oral toxicity study in rats.
Result - No toxic signs and symptoms/mortality
Acute Dermal Irritation Study on Rabbits -
Result - Non - Irritant
Skin Sensitization study in Guinea pigs
Result - Non - Sensitizer.
Acute Eye Irritation study on Rabbit -
Result - Non Irritant

Procedure

Measure your blood glucose just two hours after lunch , if the reading is more than 140 mg/dl (normal value) and lies between 160mg/dl to260 mg/dl then take 1 ml of the oil, and rub between knee to ankle portion over both the legs. In case the reading is in the range of 260mg/dl to 360mg/dl then use 2 ml of the oil and if the range is between 360 to 460mg/dl, then use 3 ml of the oil. Wait for two and half an hour and once again measure your blood glucose which will be found to become normal. Never eat or drink anything except water between lunch and the time of second reading measurement (four and half an hour). During herbal oil treatment, one should strictly follow the calorie chart till the glucose level is not maintained. Diabetes herbal oil is not to be taken orally.

Conclusion

Thus when sugar level is high, patients must choose such meal which comprises Moong dal, Cabbage as vegetable and Roti, made from mixture of maize, soya bean and wheat flour so that they can consume minimum carbohydrates. Since the oral anti diabetic drugs and artificial insulin4 are associated with the side effect of hypoglycemia due to the presence of amino functional group in their molecule therefore, it is a misconception that any oral anti diabetic drug, stimulate the pancreas, to make natural insulin to control high blood sugar level or artificial insulin can work in the same pattern of natural insulin.

On an average 1800-2200 and 1200-1400 calories are required for an average male and female respectively for staying fit These calories are mainly contributed by Sugars, Fats , Proteins, carbohydrates and dietary fibers. But eating habits of us is slightly unorganized and we generally consume more calories than required. It is impossible to transform glucose into energy without physical activities. Therefore diabetics should not completely depend on drugs instead they should create awareness about the calories intake and calories utilized while trying to normalize their high blood glucose using herbal oil. To regulate the functioning of their pancreas and to undo insulin resistance, while using herbal oil5, patients must consult calorie/blood sugar, chart and should avoid food items made of grain flour and prefer pulses and green vegetables because grains flour have high % of carbohydrates and less proteins but pulses have less carbohydrates and more proteins. Also during diabetes oil treatment6, patients who are unable to do physical activity /walking, should completely avoid sugar, potato, rise, milk and oil, which are mainly responsible for increasing blood glucose level abruptly. The toxicology study7 report from Shri Ram Institute for Industrial Research for the diabetes herbal oil was accorded favorable and clinical trial report was absolutely satisfactory (table-2).

Table-2 Herbal Oil Controlling Blood Sugar - Sample Test Results

Patient Name	Date	Before Massage					After Massage		
		No. of Drops Massaged	No. of Oral Tablets Taken	Units of Insulin taken	Post Prandial Plasma Glucose mg/dl	Fasting Plasma glucose mg/dl	Post Prandial Plasma Glucose mg/dl	Fasting Plasm glucose mg/dl	Ref
Rajnish Kumar	4/3/2011	25	3	x	334	x	225	x	1
Rajnish Kumar	5/6/2011	40	3	x	x	140	x	101	1
Sangeeta Srivastava	4/3/2011	10	1	40	387	x	191	x	2
Sangeeta Srivastava	5/6/2011	10	1	40	377	x	190	x	2
Mukesh Kumar	6/22/2012	20	2	x	x	132	x	112	3
Mukesh Kumar	7/4/2012	35	2	x	260	x	120	x	3
Usha Srivastava	7/14/2012	30	2	5	240	x	144	x	4
Manju Saxena	3/10/2012	80	1	18	567	x	338	x	5
Manju Saxena	8/10/2012	55	1	15	x	205	x	100	5
Akhilesh kumar	6/22/2012	80	2	x	x	252	x	180	6
Akhilesh kumar	7/12/2012	46	2	x	280	x	120	x	6
Raj Kumari Garg	5/1/2011	20	2	40	310	x	290	x	7
Raj Kumari Garg	6/1/2011	20	1	25	290	x	213	x	7
Raj Kumari Garg	7/1/2011	20	1	25	213	x	202	x	7
Raj Kumari Garg	8/1/2011	20	1	25	202	x	152	x	7
Raj Kumari Garg	9/1/2011	20	1	25	x	130	x	105	7
Indumohan	2/20/2012	40	2	x	228	x	107	x	8
Indumohan	3/21/2012	20	2	x	x	120	x	102	8
Sangeeta Manocha	7/16/2012	80	2	x	x	180	x	100	9
Sangeeta Manocha	7/24/2012	40	2	x	300	x	155	x	9
M A Khan	5/8/2011	80	1	24	442	x	268	x	10
M A Khan	6/14/2011	60	1	24	x	160	x	110	10
K. K. Pandey	7/22/2012	30	2	x	230	x	139	x	11
K. K. Pandey	7/27/2012	30	2	x	x	130	x	95	11
Vimal Shankar	7/22/2012	05	x	x	156	x	90	x	12
Vimal Shankar	7/29/2012	33	x	x	260	x	115	x	12
Vimal Shankar	8/8/2012	15	x	x	x	115	x	102	12
Deepak Kumar	7/27/2012	55	5	x	300	x	102	x	13
Deepak Kumar	8/5/2012	50	x	x	x	150	x	110	13
Deepak Kumar	11/8/2012	40	5	x	256	x	144	x	13
Nishi Gupta	7/15/2012	15	x	x	152	x	106	x	14
Nishi Gupta	7/29/2012	15	x	x	x	115	x	97	14
Mohd Mehandi	7/30/2012	30	2	x	260	x	160	x	15
Pratima Srivastava	7/16/2012	50	2	z	284	x	196	x	16
Pratima Srivastava	7/26/2012	36	2	x	x	145	x	100	16
Ashok kumar	1/26/2012	40	2	x	190	x	100	x	17
Ashok kumar	2/7/2012	50	2	x	240	x	123	x	17
Ashok kumar	7/2/2012	57	2	x	x	157	x	85	17
Ashok kumar	8/3/2012	40	2	x	260	x	144	x	17
Gayatri Devi	7/22/2012	70	2	10	354	x	200	x	18
Gayatri Devi	7/29/2012	20	2	10	x	120	x	101	18
Manoj Srivastava	4/4/2011	80	4	x	220	x	143	x	19

Manoj Srivastava	4/13/2011	20	2	x	298	x	200	x	19
Manoj Srivastava	5/20/2011	20	2	x	x	120	x	102	19
P N Shukla	7/16/2012	30	6	x	215	x	111	x	20
P N Shukla	8/3/2012	20	6	x	x	120	x	102	20
Manju Gupta	7/2/2012	40	2	x	300	x	180	x	21
Manju Gupta	7/12/2012	90	2	x	x	190	x	105	21
Manoj Johri	7/6/2012	40	4	x	180	x	117	x	22
Manoj Johri	7/12/2012	15	4	x	x	115	x	102	22
Mamta (Kidney Pat.)	7/5/2012	14	2	x	x	109	x	96	23
Mamta (Kidney Pat.)	7/16/2012	10	2	x	170	x	142	x	23
Sudha Bhartiya	7/7/2012	40	x	x	260	x	120	x	24
Sudha Bhartiya	8/8/2012	20	x	x	x	120	x	99	24
R K Vishnoi	4/8/2011	80	1	x	254	x	155	x	25
R K Vishnoi	7/5/2012	47	2	x	280	x	171	x	25
R K Vishnoi	7/5/2012	47	2	x	x	147	x	102	25
Ajay Kumar Dubey	7/19/2012	35	1	x	240	x	142	x	26
Ajay Kumar Dubey	7/28/2012	30	1	x	x	135	x	107	26
Kavita Saxena	4/4/2011	40	x	x	x	140	x	100	27
Sanjai Saxena	2/2/2011	30	x	x	260	x	162	x	28
R M Maurya	7/19/2012	10	x	x	166	x	120	x	29
R M Maurya	7/27/2012	40	x	x	260	x	112	x	29
R M Maurya	8/4/2012	18	x	x	x	118	x	90	29
K K Maurya	7/19/2012	15	x	x	162	x	96	x	30
K K Maurya	7/27/2012	40	x	x	240	x	125	x	30
K K Maurya	8/4/2012	10	x	x	x	110	x	98	30
Sudhir Gupta	7/6/2012	40	x	x	300	x	150	x	31
Atul Kumar	7/24/2012	30	2	x	260	x	180	x	32
Atul Kumar	7/31/2012	20	2	x	x	120	x	99	32
Ajab Shukla	7/28/2012	66	2	x	340	x	140	x	33
Ajab Shukla	8/3/2012	30	2	x	x	135	x	102	33
Ajab Shukla	8/11/2012	45	2	x	262	x	100	x	33
Satya Singh	3/16/2012	20	1	x	200	x	140	x	34
Satya Singh	4/8/2012	80	x	x	x	180	x	105	34
Radha Krishnan	5/28/2012	40	x	x	234	x	180	x	35
Radha Krishnan	7/8/2012	96	x	x	x	196	x	141	35
Radha Krishnan	7/16/2012	80	x	x	x	180	x	102	35
Sudha Gupta	4/6/2011	160	x	x	600	x	300	x	36
Sudha Gupta	8/8/2011	40	x	x	x	150	x	110	36
G C Srivastava	7/28/2011	80	0.5	x	x	127	x	110	37
G C Srivastava	8/20/2011	30	0.5	x	x	130	x	105	37
G C Srivastava	9/10/2011	40	0.5	x	240	x	98	x	37
Shiv saran	4/15/2012	20	x	x	x	120	x	100	38
Vineta Dhawan	12/4/2011	80	5	x	310	x	180	x	39
Vineta Dhawan	12/5/2011	80	5	x	255	x	160	x	39
Vineta Dhawan	7/2/2012	60	5	x	x	160	x	102	39
Man Mohan	4/4/2012	30	2	45	220	x	107	x	40
R.C.Gupta	6/10/12	50	2		275		135		
R.C.Gupta	15/09/12	30	2		180		120		
Mrs.R.C. Gupta	6/10/2012	50	nill		500		350		
Asha Gupta	15/10/2012	50	3	40	350		200		
pankaj Malhtra	22/9/12	70	2		300		99		
Aruna Saxena	10/8/2012	50	2		214		150		

Aruna Saxena	12/9/2012	50	2		270		145		
Dr.Mena Singh	15/9/12	40	2		254		190		
Dr.Mena Singh	26/9/2012	70	2		258		123		
Dr.Mena Singh	3/10/2012	50	2		195		116		
Mohd.Jawaid Naiyer	8/9/2012	25	2		176		155		
Laxmi Kant Awasthi	10/9/2012	50	2		164		140		
Laxmi Kant Awasthi	24/9/12	50	2		240		154		
Mamta Morya	24/8/2012	30	x		227		140		
Asha Gupta	21/10/2012	80	2	x		230		180	
Hans Raj Gupta	25/10/2012	50	2	40		150		135	

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Study of industrially important phytochemicals of Lantana weed

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Abstract - India has a rich tradition of plant based knowledge in health care and industrially application. Among the large number of plants, weeds existing in India, very few have been studied systematically so far. Lantana is a rugged evergreen shrub from the tropics. Lantana is a Weed of National Significance. It is regarded as one of the worst weeds throughout the world because of its invasiveness, potential for spread, and economic and environmental impacts. Lantana camara L. is regarded both as a notorious weed and a popular ornamental garden plant and has found uses to cure various diseases and production of various commercially important products in many parts of the world. Lantana also produces a number of metabolites in good yields and some have been shown to possess useful biological activities. All these aspects are considered in this short review to allow an evaluation of the potential for utilization of the large biomass of Lantana available.

Introduction

The genus Lantana Verbenaceae as described by Linnaeus in 1753 contained seven species, six from South America and one from Ethiopia [1]. Lantana from the Latin lento, to bend probably derives from the ancient Latin name of the genus Viburnum which it resembles a little in foliage and inflorescence. Lantana is mostly native to subtropical and tropical America, but a few taxa are indigenous to tropical Asia and Africa. The recorded number of Lantana species varies from 50 to 270 specific and subspecific entities, but it appears that a better estimate is 150 species. The genus is a difficult one to classify taxonomically since species are not stable and hybridisation is widespread, shape of inflorescence changes with age, and flower colours vary with age and maturity [1]. Lantana camara is the most widespread species of this genus, growing luxuriantly at elevations up to 2000 m in tropical, sub-tropical and temperate regions [2]. The species name, camara, is probably adopted from the West Indian colloquial name for the common species [3]. It is a woody straggling plant with various flower colours, red, pink, white, yellow and violet. The stems and branches are sometimes armed with prickles or spines.

Lantana camara originates from tropical and subtropical America and is now classed as a serious weed in more than 60 countries. It is considered a major problem to agriculture where it occurs in East Africa, Fiji, Hawaii, India, the Philippines, South Africa and Zambia and there are currently more than 650 hybrid varieties identified. Consequently, lantana is considered to be one of the ten worst weeds worldwide.

Lantana camara L commonly known as weed or red sage, unniceeti (Tamil), pulikampa (Telugu) and caturang (Hindi) is a significant weed commonly found throughout in India [4]. It is ever green strong smelling shrub, with stout recurred prickles, leaves opposite, acute or sub acute, crenate - serrate, scab rid on both side [5]. It is grew up to a height of 1-2 m, occurs luxuriously in elevation up to 2000 m in tropical [6], sub tropical and temperate regions. It was introduced in India as an ornamental plant but entirely naturalized and found throughout India, it was listed one of the important medicinal and industrial important plants of the world [7].

The fruits are useful in fistula, pustules, tumors and rheumatism [7-10]. The essential oil of Lantana camara showed a wide spectrum of antibacterial, antimicrobial and antifungal activity [11-13]. Since very long time Lantana camara. L reported to be used in traditional medicine system for itches, cuts, ulcers, swelling, bilious, fever, tetanus, and carminative [14] cold, head ache, uterine hemorrhage, chicken box, eye injuries, whooping cough, asthma, bronchitis and arterial hypertension [15,16].

Forest Research Institute, Dehradun has made an research efforts for utilization of Lantana camara was examined and optimized for production of various industrially important chemically modified products from ?-cellulose (isolated from Lantana camara) such as water-soluble carboxymethyl cellulose (DCS, LC and PH), cyanoethylcellulose (DCS) and water-soluble hydroxypropyl cellulose (DCS and PH).

The approximate total biomass produced by Lantana camara per year ranges from 15 to 17 tonnes/ha [17], which projects the availability of Lantana biomass in huge quantity. Therefore, the abundance of Lantana camara biomass (lignocellulosic material) is likely to offer a potential feedstock for ethanol production. Forest Research Institute, Dehradun has also explored the feasibility of the utilization of the Lantana camara lignocellulosic biomass for production of bioethanol was examined.

Lantana camara a noxious weed, has posed serious threat to the ecology and demands concerted efforts for its management. Utilization of its abundantly available lignocellulosic biomass, an incompetitive raw material to other industries, would be a practical proportion for the management of this weed. In this paper, the utilization of industrial importance of Lantana camara is considered. The biological activities exhibited by some of the metabolites available from L. camara are discussed as well as uses of Lantana camara as cellulosic biomass for production of industrially important products is also reported herein.

Various uses of Lantana camara Plant

Ethnopharmacology

The plant has been used in many parts of the world to treat a wide variety of disorders [18]. Lantana camara found use in folk remedies for cancers and tumours. A tea prepared from the leaves and flowers was taken against fever, influenza and stomach-ache. In Central and South America, the leaves were made into a poultice to treat sores, chicken pox and measles. Fevers, cold, rheumatisms, asthma and high blood pressure were treated with preparations from the plant. In Ghana, infusion of the whole plant was used for bronchitis and the powdered root in milk was given to children for stomach-ache [19]. In Asian countries, leaves were used to treat cuts, rheumatisms, ulcers and as a vermifuge. Decoctions were applied externally for leprosy and scabies. It has been claimed that a steroid, lancamarone, from the leaves exhibited cardiotonic properties [20] and that lantamine, an alkaloid from the stem bark and roots showed antipyretic and antispasmodic properties comparable to those of quinine, but the validity of these claims has not been confirmed. From the leaves, an alkaloid fraction which lowered blood pressure, accelerated deep respiration and caused shivering in dogs was isolated and it was suggested that it may be useful in reducing fevers, and as a treatment of asthma and hypertension [21].

Antibacterial Activity

The extract of flower, leaf, stem and root of Lantana camara. L showed antibacterial activity against Escherichia coli, pseudomonas aeruginosa, staphylococcus aureus, and staphylococcus saprohiticus. The antibacterial activity was determined by disc diffusion [22, 23] method, tube dilution technique, agar well diffusion [24] method, micro distillation method . Essential oil of Lantana camara had considerable effect of antibacterial activity. It was observed that stains of E.coli and staphylococcus aureus were more susceptible [23] to essential. Lantana Camara flower extract posses strong antibacterial activity All few types' yellow, lavender, red and white Lantana camara L flowers displayed almost similar antibacterial activities. Petroleum ether root extract shown less antibacterial activity on pseudomonas aeruginosa and staphylococcus saprophiticus. The chloroform extract produced a moderate inhibition zone against staphylococcus aureus (5m). Chloroform stem extract showed inhibitory effect [24] against staphylococcus saprophiticus.

In other study, the essential oil of Lantana camara exhibited prominent antibacterial activity against all the bacterial strains tested. Gram positive Bacillus cereus, Bacillus subtilis and Staphylococcus aureus were the most sensitive strains to Lantana camara essential oil. Nevertheless, Gram negative Klebsiella pneumonia and Pseudomonas aeruginosa were not susceptible to the essential oil at lower concentration. A matter-of-fact, Gram-positive bacterium was more sensitive to the [25] essential oils than gram-negative bacteria.

Anti cancer Activity

Anti cancer effect of lantana camara root and leaf extracts against jurkat leukemia cell was investigated by MTT assay. Lantana camara leaf extract and root extract had roughly equaled anti proliferative activity on human leukemia jurkat cells. Morphological examinations indicated apotosis induction of the mechanisam of activity on jurkat cells. The lantana camara root and leaf extracts might be subjects for further fractionation and identification to find new anti cancer agents [26].

Anti fungal Activity

Lantana camara leaves evaluated for anti microbial and anti fungal activity and Lantana camara oil was also evaluated for larvicidal activity against different mosquito larvae [27]. The essential oil of lantana camara, tested against seven bacteria and eight fungi, showed wide spectrum of antifungal activities [28].

Other Pharmacological activities

The Ethanolic extract of Lantana camara ointment prepared for topical treatment on chronic crusty or acute lesions of Dermatophilosis [29]. Mosquito larvicidal activity and Phytochemical screening of methanol and ethanol extract of leaves and flowers of lantana camara larvicidal activity on III & IV instars larvae of mosquito species Aedes aegypti and Culexquinque--fasciatus had been investigated a dose dependent manner for 24 h [30]. Essential from leaves of lantana camara possesses adulticidal activity against different mosquito species that was utilized for development of oil based insecticide as supplementary to synthetic insecticides [31].

Various uses of Lantana camara Plant for industrially important products

Phytochemistry

The phytochemistry of L. camara has attracted considerable interest due to the presence of many phytochemically important constituents. The early work on Lantana spp was concerned with studies on the essential oils although they are not high yield producers; for L. camara, the maximum yield obtained by hydrodistillation from the leaves reached 0.2% and, from the flowers, up to 0.6% [32]. A study carried out on sample obtained from L. camara trees grown in Brazil had a terpene-like, leathery, fatty, and sweaty odour. The main constituents were bisabolene derivatives with only traces of monoterpenes. The sesquiterpenes present were ? -curcumene (1.5%), (E.)-nuciferal and (Z).-nuciferol (3.9%), (-).-ar-curcumen-15- al (5.6%), ?-curcumene (8%), ar-curcumene (9.7%), (-).-epi- ?-bisabolol (10%).,(-)?-curcumen-15-al (14.9%).Interestingly, the oil contained compounds (5%) which incorporate the new italicene skeleton (1), as epimers at C10, and 7.2% of the helifolene aldehydes (2), as epimers at C7 [33].

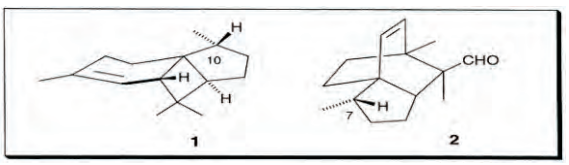

Fig.1 Source: E.L. Ghisalberti Lantana camara L. (Verbenaceae) Fitoterapia 71 (2000) 467-486

In a detailed study carried out on the toxic taxa of L. camara revealed that lantadene A and B predominated up to 2.2% of the dry weight of leaves and stem; approx. ratio 2:1[34]. In the course of these and other studies, a number of other triterpenes were identified and these are represented in Figs. 2 and 3 [35-53].

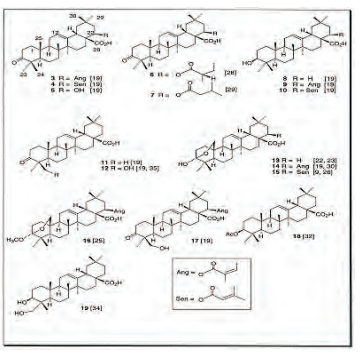

Fig.2 Source: E.L. Ghisalberti Lantana camara L. (Verbenaceae) Fitoterapia 71 (2000) 467-486

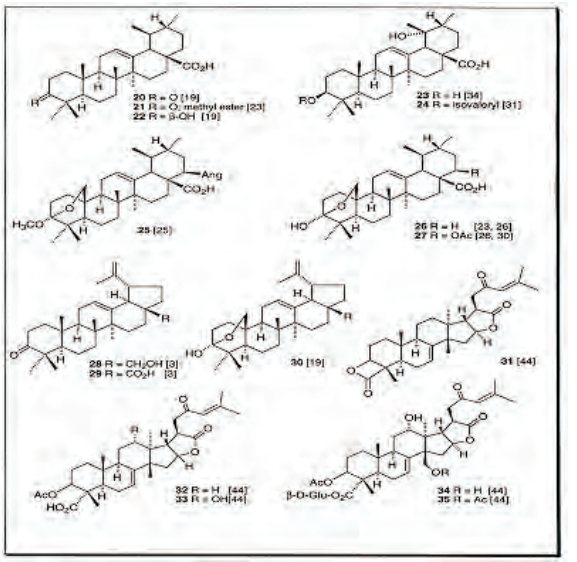

Fig.3 Source: E.L. Ghisalberti Lantana camara L. (Verbenaceae) Fitoterapia 71 (2000) 467-486

A recent investigation of the methanolic extract of L. camara has revealed a suite of euphane triterpene lactones. The presence of these metabolites, which occur in trace quantities (0.00004-0.0002%), was detected by using an assay in which thrombin activity was measured as a function of clot formation from fibrinogen [54]. L. camara grown in Sri Lanka when investigated under the study by methanol extraction it contained the quinone diodantunezone as it was previously isolated from L. achyrantifolia [55], and its regioisomer 43 [56]. The two pairs of inseparable isomers 44,46 and 45, 47 were also isolated from this extract, whereas the methanol extract of the roots contained 48 and 49 [56]

As part of a taxonomic study of Lantana, greenhouse grown plants were shown to contain the sodium salt of theveside 36 in both leaves 800 ppm and roots 900 ppm, but theviridoside 37, the corresponding methyl ester 320 ppm., was only found in the roots [57] . Also from the roots, geniposide 38, the biosynthetic precursor of theveside, has been isolated, together with 8-epiloganin 39, shanzhside methyl ester 40 and lamiridoside 41 [58,59]. The acetone wash of the leaves of L. camara contained 3-methoxy-, 3,7-di- methoxy- and 3,7,49-trimethoxyquercetin 50-52, whereas hispidulin 53 was isolated from the stems w33x. The flavone glycoside camaraside, isolated from the leaves, was originally assigned the wrong structure w51x which was later corrected to 54 [60,61]. It has also been isolated from L. camara var. aculeata together with pectolinarigenin 7-O-b-D-glucoside (55). [62].

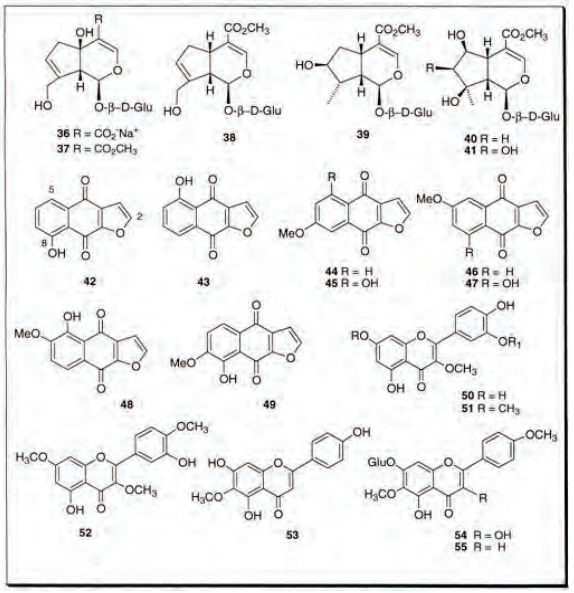

Fig.4 Source: E.L. Ghisalberti Lantana camara L. (Verbenaceae) Fitoterapia 71 (2000) 467-486

Chemical Modification of cellulose isolated from Lantana camara

Cellulose is the most abundant natural polymer from renewable sources. It is insoluble in most of organic and inorganic solvents, a major limitation of its application. To overcome this problem and to extent its application, a large number of cellulose derivative have been investigated, Derivatization of cellulose give up to trisubstituted cellulose, that three hydroxyl groups are free per glucose unit that is one primary and secondary groups which play an influential role in the preparation of cellulose derivatives [63]. Lantana camara seems to be a potential feedstock for producing alpha cellulose and its derivatives for a variety of applications having thereby a way for management of this noxious weed by its utilization into products of commercial importance. carboxymethyl cellulose, Etherication , cyanoethylcellulose (DCS) and water-soluble hydroxypropyl cellulose (HPC) from ?-cellulose is the most important routes of cellulose derivatization.

Despite the large variety of cellulose derivatives that have been made, there is continuous expansion in the world wide market of cellulose ethers because of their availability, economic efficiency, easy handling, low toxicity, and great variety of types. Combined effects of flow control, stabilization, water retention, film formation, etc. provided by cellulose ethers are not generally obtainable by the use of fully synthetic polymers. Cellulose ethers such as CMC, hydroxypropyl cellulose (HPC), cyanoethylcellulose (CEC), ethylcellulose (EC), methylcellulose (MC), Hydroxyethylcellulose (HEC), hydroxypropylmethylcellulose (HPMC), carboxy-Methylhydroxyethylcellulose (CMHEC), etc. have gained their position in the market Due to their multifunctional properties [64]. The world wide annual production of cellulose Ethers is estimated to be over 300,000 metric ton [65]. They exhibit useful properties of thickening, thermal gelation, surfactancy, film formation, and adhesion. Further they are kinetically and thermodynamically more stable and appear easy to prepare and characterize. These characteristics earn them applications in areas such as pharmacy, cosmetics, food, oil drilling, paper, paint, textiles, construction, and adhesives [65]. Among cellulose derivatives, cellulose ethers constitute the only food allowed group of modified celluloses.

Conclusion

Lantana camara L. (Verbenaceae) is a noxious weed which has imposed a great threat to land productivity, grazing livestock, biodiversity and consequently to the overall ecology. During the last few years, research has been conducted to utilize the L. camara biomass for development of furniture products, baskets, mulch, compost, drugs and other biologically active agents. Alternatively, luxuriant growth and vigorous survival make this weed of potential economic value for utilization of its abundantly available biomass into various other value added products such as bioethanol or biogas production.

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Antimicrobial Evaluation of Fruit and Leaf Extract of *Solanum nigrum*

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Abstract - Five different solvents for Solanum nigrum were sequentially used for getting their fruit and leaf extracts. Solvents were based on increasing order of polarity using soxhlet's extraction procedure. Phytochemical investigation of the extracts was carried out to ascertain constituents present in them.

All the extracts were tested for antimicrobial activity against several pathogenic/non-pathogenic microorganisms. These include 6 bacterial strains and 5 fungal strains.

Moderate antimicrobial activity was found to be present in fractions extracted from fruits of Solanum nigrum. The most potent extract of fruits was of methanol fraction having inhibitory potential against four bacterial strains used in the present study. None of the extracts from the fruits showed anti-fungal activity. In case of leaf extracts of Solanum nigrum ethyl acetate fraction was found to be most potent having inhibitory effect on four bacterial strains and two fungal strains used in present study. In general, leaf extracts of Solanum nigrum were found to possess more significant inhibitory potential against a number of bacteria and fungi.

Introduction

The herbal drug industry is growing at an astounding rate all over the world. Herbal remedies are now available not only in drug stores, but also, in food stores and supermarkets. Herbal remedies are unpurified plant extracts containing several constituents, which work together synergistically. According to WHO, herbal medicine is a defined preparation, which contains raw or processed ingredients from one or more plants, with therapeutic values. It is need of the hour to identify, isolate and purify the types of phytochemicals present in crude extracts by using modern techniques which may enhance the efficacy of these extracts against various ailments.

Solanum nigrum commonly known as "Black night shades" is a world wide weed of arable land garden. S. nigrum is however also used as leafy herb and vegetables, as fruit source as well as for various medicinal purposes like corticsteroid production.

Distribution

Solanum nigrum is a worldwide weed of arable land garden found in India, Denmark, Holland, France, Germany, Spain, Sweden, Australia and particularly widespread in Africa and South East Asia.

Vernacular Names

Commonly known as Black night shades world wide and Makoi in India it is known by various vernacular names in different languages in various countries.

In India S. nigrum is cultivated in North and South Islands, especially on cultivated and wasteland.

Chemical Composition

S. nigrum has been phytochemically investigated for the solanine ($C_{45}H_{73}NO_{15}$) content, which has been reported to be a mixture of two classes of glycosides solanines and chaconines. Alpha-solanine has been reported to be the main constituent Solanine, an alkaloidal principle, discovered by Desfosses in 1820, in the berries of S.nigrum.

Medicinal Properties of Makoi

S. nigrum has been used in folk medicine for its anti-inflammatory activity. It has both cytoprotective and cytotoxic action. In a study, S. nigrum was tested against gentamicin - induced toxicity on in-vitro cells. Cytotoxicity was significantly inhibited as assayed by Trypan blue exclusion and mitochondrial dehydrogenase activiy (MTT) assay. The test extract also exhibited significant hydroxyl radical scavenging potential thus suggesting its probable mechanism of cytoprotection (Parasnath et al., 2001), S. nigrum could be used as an anti-oxidant and cancer chemopreventive material (Son et al., 2003). Solanum nigrum L. (SNL) has been used in folk medicine for its anti-inflammatory activity. A study investigated the apoptopic signal pathway triggered by glycoprotein isolated from SNL in HT-29 cells. Activity of Allium sativum, Liliaceae plants and Solanum nigrum was tested against some common fungal species associated with superficial mycoses (Ahmed et al., 2005)

Collection of plant samples

Leaves and fruits of S. nigrum were collected from local area of Dehradun.

Processing of Samples

Leaves and fruits were dried in shade and were finally dried in oven for 3-4 hrs and then weighed and loaded in Soxhlet assembly. The material was crushed and packed in the extractor. Solvent was placed in round bottom flask and boiled. The vapours are allowed to pass through the side tube to the condenser, where they get condensed and fall back to the packed material through which it percolates and extract out the active constituents. As the volume of the solvent in the extractor increases, the level of liquid in the siphon also increases till it reaches maximum point from where it is siphoned out in the flask. Distillation is used for collection of extracts.

Qualitative Phytochemical Tests

Tests were performed to ascertain the presence / absence of different constituents in each plant extract.
The powdered form of shade dried leaves and fruits of Solanum nigrum were extracted sequentially using in soxhlet's apparatus using five solvents with increasing polarity.
The solvents used for extraction included petroleum ether, ethyl acetate, methanol, ethanol and distilled water.

Extraction and yield of soluble principles of fruits and leaves of Solanum nigrum.

Table-1a shows the amount of extracted and % yield of the soluble principles in various solvents of fruits. Table 1b shows the amount extracted and % yield of the soluble principles in various solvents of leaves of Solanum nigrum.

Table-1a - Yield of Various extracts from Fruits of Solanum nigrum

S.No.	Extraction Solvents	*Solanum nigrum*	
		Wt. of Extracts in (gms)	% Yield (w/w)
1.	Petroleum ether	7.235	4.60
2.	Ethyl Acetate	3.873	2.47
3	Methanol	9.139	5.82
4.	Ethanol	3.065	1.95
5.	Distilled Water	10.055	6.40
	Total Soluble Components		21%

Table-1b - Yield of Various extracts from Leaves of Solanum nigrum

S.No.	Extraction Solvents	*Solanum nigrum*	
		Wt. of Extracts in	% Yield (w/w)
1.	Petroleum ether	2.208	1.491
2.	Ethyl Acetate	2.152	1.454
3	Methanol	5.990	4.047
4.	Ethanol	2.308	1.559
5.	Distilled Water	9.692	6.548
	Total Soluble Components		15%

Qualitative phytochemical analysis showed the presence of different compounds in different extracts as depicted in table 2a and 2b.

Table-2a -Qualitative Chemical analysis of Fruit Extract of Solanum nigrum

Extracts	Petroleum	Ethyl	Methanol	Ethanol	Aqueous
Test Performed					
1. Test for Alkaloids					
a) Mayer's Test	+	+	+	+	-
b) Hager's Test	+	+	+	+	-
c) Wagner's Test	+	+	+	+	-
2. Test for Proteins					
a) Millon's Test	-	-	-	-	-
b) Biuret Test	-	-	-	-	-
c) Ninhydin Test					
3. Test for Triterpenoids	-	-	+	-	-
a) Salwkowski's Test	-	-	+	-	-
b) Libbermann-Burchard's Test	-	-	+	-	-
4. Test for flavanoids					
a) Ferrichloride Test	-	+	+	+	-
b) Alkaline reagent Test	-	+	+	+	-
c) Zinc hydrochloride Test	-	+	+	+	-
5. Test for Carbohydrates					
a) Molisch's test	+	-	+	+	+
b) Selvinoff's Test	+	-	+	+	+
c) Benedict's Test	+	-	+	+	+

Table-2b - Qualitative Chemical analysis of Leaf Extract of Solanum nigrum

Extracts	Petroleum	Ethyl	Methanol	Ethanol	Aqueous
Test Performed					
1. Test for Alkaloids					
a) Mayer's Test	+	+	-	-	-
b) Hager's Test	+	+	-	-	-
c) Wagner's Test	+	+	-	-	-
2. Test for Proteins					
a) Millon's Test	-	-	-	-	-
b) Biuret Test	-	-	-	-	-
c) Ninhydin Test	-	-	-	-	-
3. Test for Triterpenoids					
a) Salwkowski's Test	-	-	-	-	-
b) Libbermann-Burchard's Test	-	-	-	-	-
4. Test for flavanoids					
a) Ferrichloride Test	-	+	+	+	-
b) Alkaline reagent Test	-	+	+	+	-
c) Zinc hydrochloride Test	-	+	+	+	-
5. Test for Carbohydrates					
a) Molisch's test	-	-	+	+	-
b) Selvinoff's Test	-	-	+	+	-
c) Benedict's Test	-	-	+	+	-

Antimicrobial Potential of Solanum nigrum Leaf Extract

All the extract fractions were assessed against test organisms used. Leaf extract of Solanum nigrum showed moderate inhibitory potential against four bacterial strains and three fungal strains. Inhibition was dose dependent in efficacy against bacteria and fungi with most of the extracts showing maximum inhibition at 100% concentration. Maximum ZOI (20mm) was obtained for ethyl acetate extract against - P. aeruginosa.

Table-3a Antimicrobial Screening of Different Extract Fraction of Solanum nigrum fruits

Mean Inhibition Zone Diameter (in mm)															
Test Organisms	**Petroleum Ether Fraction**			**Ethylacetate Fraction**			**Methanol Fraction**			**Ethanol Fraction**			**Distilled Wate Fraction**		
	100 %	50 %	25 %	100 %	50 %	25 %	100 %	50 %	25 %	100 %	50 %	25% %	100 %	50 %	25 %
1. E.coli	-	-		12	10	9	15	10	9	-	-	-	-	-	-
2. S. aureus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3. B. subtilis	16	12	15	10	9	-	14	11	9	14	12	10	-	-	-
4. P. aeruginosa	-	-	-	12	11	10	21	15	10	12	10	-	-	-	-
5. S. typhimurium	-	-	-	-	-	-	11	10	9	-	-	-	-	-	-
6. S. dysenteriae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7. A. niger	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table-3b - Antimicrobial Screening of Different Extract Fraction of Solanum nigrum leaves

Zone of Inhibition : < 10mm - Low Activity
10 - 20 - Moderate Activity
> 20mm - High Activity

Mean Inhibition Zone Diameter (in mm)															
Test Organisms	**Petroleum Ether Fraction**			**Ethylacetate Fraction**			**Methanol Fraction**			**Ethanol Fraction Fraction**			**Distilled Wate Fraction**		
Bacteria	100%	50 %	25 %	100 %	50 %	25 %	100 %	50 %	25 %	100 %	50 %	25% %	100 %	50 %	25 %
1. E.coli	10	10	9	18	15	12	11	10	9	-	-	-	-	-	-
2. S. aureus	-	-	-	14	12	10	-	-	-	-	-	-	-	-	-
3. B. subtilis	12	11	10	15	12	10	-	-	-	10	9	-	-	-	-
4. P. aeruginosa	13	10	9	20	11	10	15	12	10	12	11	10	-	-	-
5. S. typhimurium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6. S. dysenteriae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fungi															
7. A. niger	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8.A. Clavitus	-	-	-	11	10	9	-	-	-	-	-	-	-	-	-
9.Penicillium spp	10	9	7	12	11	-	-	-	-	-	-	-	-	-	-
10.S. cervisae	10	9	8	-	-	-	-	-	-	-	-	-	-	-	-
11.C. albicans	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

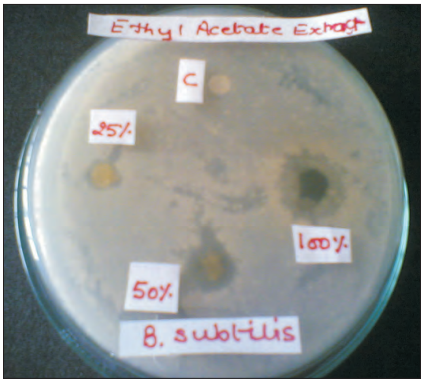

Plate-1- showing ZOI of ethyl acetate Extract of *S.nigrum* leaves against *B.subtilis*

Plate-2- Showing ZOI of ethyl acetate Extract of *S.nigrum* leaves against *E.coli*

Fig.1- Soxhlet apparatus for extraction

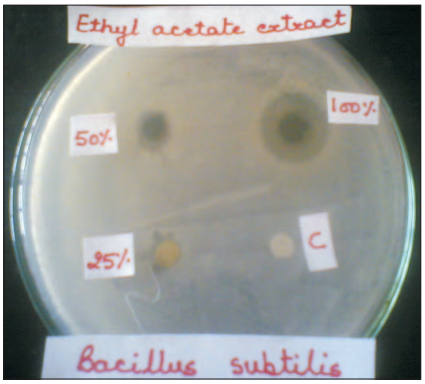

Plate-3- Showing ZOI of Ethyl Acetate Extract of *S.nigrum* fruits against *B.subtilis*

Minimum Inhibitory Concentration Testing of fruits of *Solanum nigrum.*

The methanol extract of fruits has given uniform antibacterial activity therefore it was used for determination of minimum inhibitory concentration (MIC). The results of MIC test are shown in Table-4a.

Table-4a Determination of MIC of Methanol fraction of fruits of Solanum nigrum

Test Organisms	Concentration showing ZOI (mm)				
	50%	25%	12.5%	6.25%	3.12%
1. *E.coli*	10	9	8	-	-
2. *B.subtilis*	11	9	7	-	-
3. *P. aeruginosa*	15	10	9	7	6
4. *S.typhimurium*	11	10	9	7	-

Minimum Inhibitory Concentration Testing of Leaves of Solanum nigrum.

The ethyl acetate extract was found to have maximum inhibitory potential, at 25% concentration, therefore, it was used for determination of minimum inhibitory concentration (MIC). The results of MIC test are shown in Table-4b.

Table-4b - Determination of MIC of Ethyl acetate fraction of leaves of *Solanum nignum*

Test Organisms	Concentration showing ZOI (mm)			
	50%	25%	12.5%	6.25%
1. *E.coli*	15	12	9	6
2. *S. aureus*	12	10	8	-
3. *B.subtilis*	12	10	7	-
4. *P. aeruginosa*	11	10	7	6

It is evident from the results that: Methanol extract of fruit is found to be most active against four bacterial strains. MIC of this extract reveals that it is active even at a very low concentration (3.12%). This may be due to triterpenoids present only in methanol extract in addition to other constituents such as alkaloids and flavanoids, which may also be responsible for its antimicrobial activity.

Moderate activity observed in the methanol extract of leaves could be due to absence of alkaloids & triterpenoids in this extract.

Similar results were shown by Rani and Khullar, 2004, with the methanol extract of this plant.

In the leaf extract, ethyl acetate fraction is the most potent one having inhibitory potential against four bacterial and two fungal strains. This could be due to presence of akaloids and flavanoids in the extract.

Compared to this the Ethyl acetate extract from fruits did not show potent inhibitory activity.

It is apparent from the present comparative analysis of its leaves & fruits, that leaves can be more useful in controlling microbial infections. Solanum nigrum is an easily available plant in India. The component present in the leaf fraction can be further isolated, analysed and used as a potent antimicrobial agent.

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Effect of Phytohormones on in vitro Growth of *Centella asiatica Linn.*

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Abstract - The present investigation aims at developing rapid micro propagation protocol, which can be used for conservation of Centella asiatica and mass multiplication of a valuable medicinal plant to meet out the pharmaceutical demand and its conservation. Attempts were made to evolve a rapid in vitro technology to conserve this valuable medicinal herb in very short duration. The combinations of BAP (4.0 mg/l & 5.0 mg/l) with IBA (0.25 mg/l) showed shoot proliferation (83.3±0.16 %). The combination of BAP with IBA (2:0.25) in MS media showed maximum shoot elongation (2.25±0.70). Among the combinations of BAP with IBA & NAA, MS media supplemented with BAP: NAA (4:0.25) showed maximum number of shoots per explants (1.2±0.38). BAP (4.0 mg/l) with NAA (0.4 mg/l) showed highest (6.13±0.16) multiplication rate. MS media supplemented with BAP with IBA (4:0.5 & 5:0.5) resulted in shooting as well as rooting simultaneously. Micro propagated plantlets were hardened, acclimatized and transferred to the field.

Biochemical investigation revealed significantly higher total sugar as well as protein contents in in vitro raised plants than field grown plants however total starch content was lower in micropropagated microshoots..

This micropropagation procedure could be useful for mass multiplication of superior plant material for field cultivation as well as research purpose.

Key words- Axillary shoot proliferation, node culture, axenic, microshoots, BAP, NAA, IBA.

Introduction

In India, approximately 1700 plant species are used in ayurveda, 500 for Siddha, 400 for Unani, 300 for Amchi systems of medicine with substantial overlaps of common plants among these sys-tems. Centella asiatica (Bhrami in Hindi; Vallarai in Tamil) is a small herbaceous annual plant of the family Apiaceae,and is native to India. It is used as a medicinal herb in Ayurvedic medi-cine for increasing memory power.The plant enjoys considerable reputation in Indian system of medicines as a brain tonic.

It is also used in treatment of asthma, bronchitis, dropsy, elephantiasis, gastric catarrh, kidney troubles, leucorrhoea, skin disease and urethritis .It has antibacterial, anti-feedent, anti- filarial, anti- stress, anti-tuberculosis activities and wound healing properties Chakroborty et al., (1996); Srivastava et al., (1997).

In India, Centella asiatica, the plant frequently suffers due to growing modern agriculture, in-creasing use of herbicides, drastically depleting water level in river, canals and irrigation channels or adding of sewage water in the river cause rapidly eroding natural habitat. The species are facing extinction in natural habitat, to cope up with this adverse condition, the in vitro propagation is a boon for mass production and conservation of this valuable medicinal herb.

Earlier in vitro propagation through callus cultures [Josekutty (1998), Patra et.al (1998), Rao et.al], axil-lary buds [Ramshree et.al (2004), Tiwari et.al (2004)] , shoot tips [Sangeetha et. al (2003)], leaf explants [Banerjee et. al (1999)], stolons [Sampath et. al (2001)] and somatic embryogenesis [Martin (2004), Paramageetham et. al (2004)] were reported in Centella asiatica. The present investigation aims at developing a rapid protocol, which can be used for large scale production and conservation of Centella asiatica to meet the pharmaceutical demand.

Materials And Methods

Explant source

Nodal explants of Centella asiatica were collected from medicinal garden of FRI Dehradun. Nodal segments (2.5-3.0cm.) were prepared with the help of sharp secateurs and washed thoroughly under running tap water to remove the dust particles followed by washing in liquid detergent (labolein) solution with 5-10 drops/100ml of Teepol (Glaxo India Ltd, Mumbai, India) for 5 min and then presterilized in a mixture of 1% Bavistin and 1% Blitox solution for 15 min. The explants so treated with fungicide were then washed three times with sterile water before sterilizing in 0.1% (w/v) $HgCl_2$ for 3 minutes. To remove the traces of sterilant, explants were rinsed vigorously in sterile water at least three times. Prior to inoculation proximal and distal ends of explants were trimmed to reduce them to 15-20 mm. size.

Culture condition

Liquid Murashige and Skoog (MS) basal medium (consisting of salts, vitamins and 3 % sucrose) was used

for inoculation. Different plant growth regulators (PGRs) viz. 6-benzylaminopurine (BAP), indole-3-butyric acid (IBA) and Naphthalene acetic acid (NAA) were added at various concentrations to MS medium before the pH of the medium was adjusted to 5.6. Media were autoclaved at 1.06 kgcm?2 and 121 ˚C for 15 min. Cultures at all growth stages were incubated under artificial conditions: 25±2 ˚C, 60 % RH and a 16-h photoperiod (using white fluorescent tubes) under a photosynthetic photon flux density of 40 ?molm?2 s?1.

Axillary bud initiation and multiple shoot proliferation

The explants were inoculated in solid MS medium with different concentrations (1-5 mg/l) of BAP along with different concentrations of IBA and NAA in tissue culture tubes. A minimum of 24 replicates were maintained for each treatment. Within 10 days, buds were induced in incubated cultures. Elongated axillary buds afetr 15 days were separated and cultured for multiple shoot proliferation on the same medium. For further shoot multiplication, five varying levels of NAA (0.1-0.5 mg/l) supplemented with 4 mg/l BAP were attempted. The control was devoid of PGRs.

Data recording

Data was recorded in respect to multiple bud induction (Number of multiple buds, percentage of cultures responding). Only those explants which formed 3 or more than three buds in a single preformed axillary bud were scored for multiple buds and average number of buds induced was calculated by subtracting existing axillary buds at the time of inoculation. For axillary shoot proliferation, data was recorded in respect of number of axillary shoots/explant, conversion percentage of buds into shoots (total number of shoots/total number of buds x100) and length of induced shoots. Data of multiplication rate was recorded in respect to gain in number of shoots per subculture cycle (increase in number of shoots= number of shoots induced- number of shoots cultured) and dividing the gain by number of shoots in a propagule. The shoots obtained on these concentrations were subcultured on medium having same composition for further multiplication.

Subculturing

Subculturing was done at 15 days interval. For this a clump of 3-4 shoots was subcultured on the same medium and the effect of subsequent subculturing was observed on the multiplication of shoots.

Root induction and elongation

Roots were induced during bud induction as well as multiplication since media were already supplemented with auxins. The plantlets with well-developed rhizosphere and rhizome system were advanced for acclimatization.

Acclimatization

A simple acclimatization process was established to make the most of the survival rate. Prior to the core part of acclimatization, the well-rooted plantlets were kept in incubation room in plastic/ Thermocol pots filled with sterilized vermiculite (coarse grain) under artificial condition and allowed to grow for one week. Rooted planlets were trasferred to mist chamber with a transparent cover of polythene sheet for 20 days, with good aeration and high humidity. The partially acclimatized plantlets were shifted in a shade house in earthen pots containing soil mixture (soil, sand and farm yard manure in 1:1:1 ratio). Over the next six months these plants developed profuse rhizome and root system.

Experimental Design And Statistical Analysis

The experiment was designed in a randomized design. Each treatment carried 24 ramets. The experiment was replicated thrice. The recorded numerical information aided in determining the standard error. The data collected was subjected to the analysis of variance (ANOVA) and significant differences among the treatments were tested by Duncan's multiple range test (Duncan 1955) at 5 % level using SPSS (Version 16).

Results And Discussion

In vitro shoot induction

For present study nodal explants was taken for shoot induction Nodal segments of mature plants have been however used in most cases. Nodal explants of B. Monnieri were propagated in vitro using shake cultures Tiwari et al., (2000); nodal explants were also used for Eclipta alba Gawdwa and Paratkar, (2004); shoot tip, nodal and intermodal segments were reported in phyllanthus amarus Ghanti et al., (2004). Stem and leaf explants of green house green plants were used for the regeneration from callus cultures of Centella asiatica, Patra et al.,(1998). Banerjee et al., (1999), used 5-6 month old glass house grown plants of Centella asiatica for in vitro multiplication from leaf explants Tiwari et al., (2000) has reported micropropagation of Centella using

nodal segments for clonal propagation of C. asiatica.

The data pertaining to the effect of different combinations of BAP and auxins viz. IBA & NAA on shoot induction and proliferation of Centella asiatica in a two-way factorial experiment is presented in Table-1. Maximum bud break (83.33±0.16) was achieved in BAP 5mg/l &4mg/l + IBA 0.25mg/l. The treatment of BAP and auxins in combination and their interactions had statistically significant effects (P > 0.05) on the rate of shoot proliferation. In all the treatments, the shoot induction increased from lower to higher concentration i.e., 5mg/l &4mg/l of BAP. Significant variable rate of shoot proliferation was obtained in different concentrations of BA. The maximum shoot induction (90%) was observed with BAP 5mg/l & 4mg/l + IBA 0.25mg/l as well as 4 mg/l BAP+ 0.5 mg/l IBA, which was significantly higher than other combinations. Maximum shoot proliferation through callus culture was obtained on MS medium supplemented with 2.0 mg/l kinetin and 4.0 mg/l a-napthaleneacetic acid by Patra et al., (1998) in C. asiatica.

The treatment of IBA (0.5 mg/l) along with BAP resulted in on shoot as well as root induction whereas other combinations were failed to induce rooting at very preliminary phase. Maximum shoot number per explant (1.2±0.38) was recorded when MS media was supplemented with 4 mg/l BAP + 0.5 mg/l NAA followed by 4 mg/l BAP + 0.5 mg/l NAA (1.1±0.35) which were significantly higher than others while maximum shoot length (2.25±0.70) was observed on MS media supplemented with 2.0 mg/l BAP + 0.25 mg/l of IBA which was significantly higher than others. Rooting was achieved on two combinations viz. 4.0 mg/l BAP + 0.5 mg/l IBA (1.25 ±0.25) & 5.0 mg/l BAP + 0.5 mg/l IBA (2.0 ±0.63). For multiplication of shoots further, subculturing was performed on MS medium supplemented with combinations of BAP (4mg/l) & varying concentrations of NAA (Fig.1(A-D)).

Shoot multiplication

Shoot multiplication was done on MS media supplemented with BAP & lower concentration of NAA (0.1 to 0.5 mg/l).Although the initial sprouting required the presence of BAP at 2 mg/l concentration and IBA at 0.1 mg/l concentration, differentiation of shoots and their growth required transfer of cultures to a medium with relatively higher concentration of BAP and NAA at a lower concentration instead of IBA (Banerjee et al.1999) The data pertaining to the effect of different combinations of BAP and NAA on the shoot multiplication of Centella asiatica in a two-way factorial experiment is presented in Table-2. Different concentrations of NAA had different effects on the rate of shoot multiplication. The treatment NAA 0.4mg/l had statistically significant effects (P < 0.05) on the rate of shoot multiplication. It was observed that the maximum rate of shoot multiplication (6.13±0.16) was obtained on medium supplemented with 4.0 mg/l of BAP & 0.4mg/l of NAA, which was significantly higher than the shoot multiplications obtained with other NAA combinations, in three weeks period (Fig.2(A, B & C)).

The longest shoots (5.2±0.10) were obtained on the same medium i.e., 4.0 mg/l of BAP & 0.4mg/l of NAA which was significantly higher than other treatments. Significant decrease in mean shoot length was observed upon the addition of NAA beyond 0.4 mg/l (Table-2).

In vitro rooting of microshoots

In vitro rooting was archived during shoot induction therefore same had been followed for rooting. Maximum mean root number 2.0 ±0.63 , was achieved on IBA 0.5mg/l along with 5 mgl/ BAP supplemented full strength MS media. A well developed root system obtainable after three weeks of inoculation of nodal explants. (Fig.1(E & F)).

Hardening, acclimatization

The plantlets with well developed root and shoot systems were transferred to a 500 ml glass jars containing sterilized vermiculite presoaked with half strength MS nutrient medium (Sucrose-free). The jar bottles were initially kept for two weeks in culture room. More than 90% of the plantlets survived when subjected to two weeks hardening in the culture room. These plantlets were shifted to plastic pots containing soil, sand, and FYM (farmyard manure) in 1:1:1 ratio and were placed in the mist chamber for 20 days and subsequently shifted to an open shade house for acclimatization to external environment (Fig.3(A & B)). The plantlets were then transplanted in polybags containing same soil mixture and kept under shade house. Over the next six months, these plantlets showed a well developed rhizome and root system. Gradually the plantlets were exposed to natural conditions.

Field transfer

The plantlets hardening were shifted to the field at Plant physiology discipline, FRI Dehradun. They were planted in randomized block design for their performance.

Table-1 - Effect of different combinations of BAP, IBA & NAA on percent shoot induction, number of shoots per

explant length of shoots (cm) and Root length (cm) from nodal segments of Centella asiatica cultured on MS media. (Observations recorded after 2 weeks of inoculation).

Treatment	Concentration (mg/l)	Shoot induction (% ± SE)		Number of shoots per explants (Mean ± SE)		Shoot length (cm) (Mean ± SE)		Root length (cm) (Mean ± SE)
Control	-	-			-	-	-	
BAP+IBA	1:0.25	33.3	±0.21a b	0.4	±0.25 ab	0.98	±0.62 abcd	-
	2: 0.25	50.0	±0.22a b	0.5	±0.23 ab	2.25	±0.70d	-
	3: 0.25	50.0	±0.22a b	0.56	±0.25 ab	1.93	±0.86cd	-
	4: 0.25	83.3	±0.16b	0.59	±0.11 ab	1.3	±0.26 ab	-
	5: 0.25	83.3	±0.16b	0.58	±0.11 ab	0.87	±0.18 abcd	-
BAP+IBA	1:0.5	-	-	-	-	-	-	-
	2: 0.5	-	-	-	-	-	-	-
	3: 0.5	-	-	-	-	-	-	-
	4: 0.5	83.3	±0.16b	0.4	±0.08 b	1.6	±0.32bcd	1.25 ±0.25b
	5: 0.5	66.7	±0.21b	0.5	±0.16 ab	1.4	±0.45 abcd	2.0 ±0.63c
BAP+ NAA	1:0.25	33.3	±0.21ab	0.45	±0.28 ab	0.54	±0.34 abc	-
	2: 0.25	33.3	±0.21ab	0.62	±0.39 ab	0.71	±0.45 abc	-
	3: 0.25	50.0	±0.22ab	0.72	±0.32 ab	0.33	±0.15 ab	-
	4: 0.25	66.7	±0.21b	0.95	±0.30 ab	0.25	±0.08 ab	-
	5: 0.25	66.7	±0.21b	0.62	±0.20 ab	0.83	±0.26 abc	-
BAP+ NAA	1:0.5	33.3	±0.21ab	0.35	±0.22 ab	1.1	±0.69 abcd	-
	2: 0.5	33.3	±0.21ab	0.50	±0.32 ab	0.9	±0.57 abcd	-
	3: 0.5	50.0	±0.22ab	0.98	±0.44 b	1.3	±0.60 abcd	-
	4: 0.5	66.7	±0.21b	1.2	±0.38 b	0.65	±0.20 abc	-
	5: 0.5	66.7	±0.21b	1.1	±0.35 b	0.4	±0.13 ab	-
CD (0.05) treatment		**0.0095**		**0.062**		**0.025**		
CD (0.05) concentration		**0.016**		**0.108**		**0.013**		

Data shown are Mean ± SE of three replicates, each replicate consisted of 24 ramets. Mean followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

Table-2 Effect of different combinations of BAP & NAA on rate of multiplication and length of shoots (cm) from propagules* of Centella asiatica cultured on MS media. (Observations recorded after six weeks of culture on MS media).

Treatment	Concentration (mg/l)	Shoot length (cm) (Mean ± SE)		Multiplication Rate (Mean ± SE)		Shoot No. (Mean ± SE)	
CONTROL	-	1.05	±0. 07a	1.05	±0. 07 a	1.46	±0. 17 a
BAP+ NAA	4:0.1	3.43	±0.11b	2.11	±0.05b	2.0	±0. 07 b
	4: 0.2	3.8	±0.13 b	3.43	±0.22 c	3.7	±0.10 c
	4: 0.3	4.38	±0.24 c	5.36	±0.19 d	4.16	±0. 09 d
	4: 0.4	5.2	±0.10 d	6.13	±0.16 e	5.33	±0.15 f
	4: 0.5	3.66	±0.12 b	5.08	±0.13 d	4.55	±0. 11e
CD (0.05)		0.171	0.356				

Data shown are Mean ± SE of three replicates, each replicate consisted of 24 ramets. Mean followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

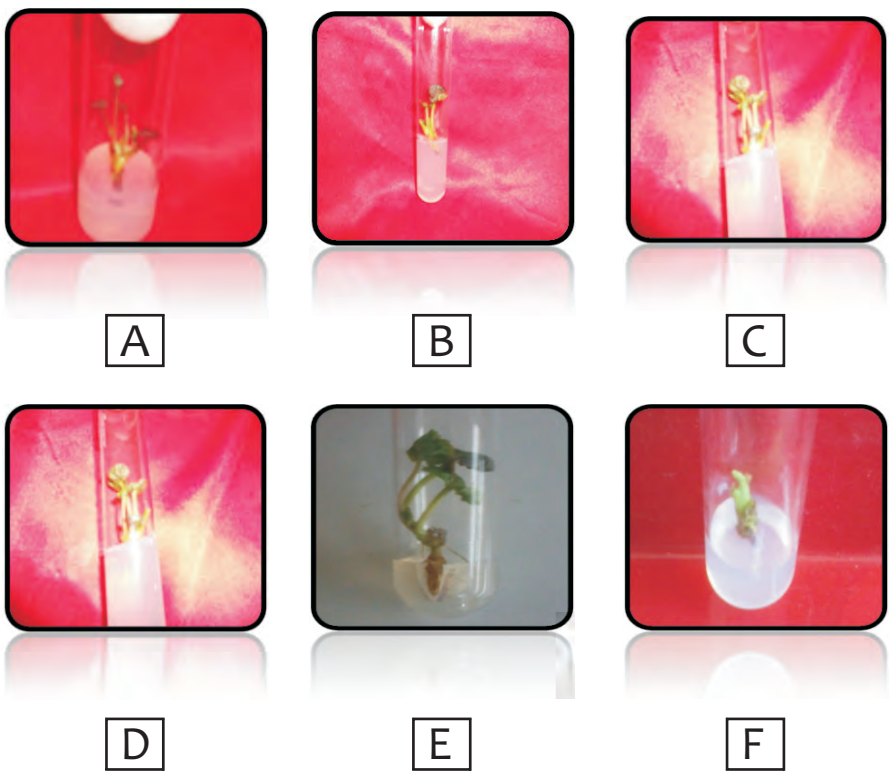

Fig.1(A & D) - Bud break in nodal explants of C. asiatica, Fig.E & F: shoot as well as root induction in nodal explants of C. asiatica

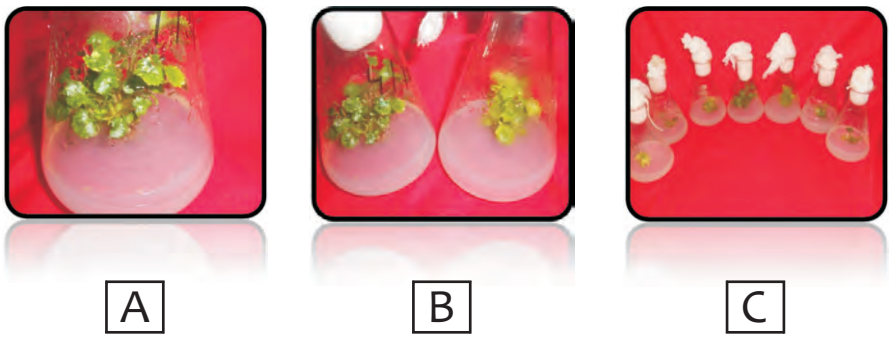

Fig.2 (A, B & C) - Multiplication (2nd subculture) of microshoots of C. asiatica

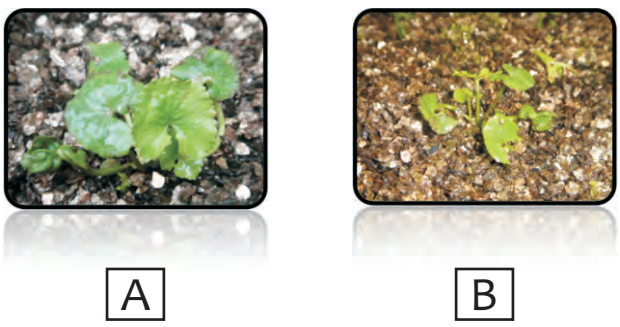

Fig.3 (A & B) - Hardened & Acclimatized plantlets of C. asiatica under shade house

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Chemical Examination of The Leaves of *Pavetta indica* And *Scindapsus officinalis*

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Abstract - Chemical examination of the leaves of two medicinally important plants Pavetta indica and Scindapsus officinalis was carried out. Five compounds including two triterpenes (?- amyrin and ursolic acid), one flavonoid (quercetin-3-O-galactoside), and two phenolic acids (caffeic acid and p-hydroxybenzoic acid) were characterized in the leaves of Pavetta indica while the leaves of Scindapsus officinalis were found to contain five compounds consisting of four flavonoids (quercetin, rutin, dihydroxykaempferol and cyanidin), and one triterpene (?- sitosterol). Identification of ursolic acid and quercetin -3-O-galactoside in Pavetta indica and quercetin, rutin, dihydrokaempferol, ?-sitosterol and cyanidin in Scindapsus officinalis is the first report of their occurrence in these plants.

Keywords: Pavetta indica, Scindapsus officinalis, chemical constituents

Introduction

Chemical prospecting of biodiversity is important in light of the threat to ecosystem's productivity in general and to health care in particular presented by ever increasing pressure on our resources and toxic effects and resistance associated with the existing drugs. Medicinal plant materials are used throughout the world as home remedies over the counter drug product and raw material for the pharmaceutical industry and represent a substantial proportion of the global drug market. An efficient management and sustainable use of medicinal plant resources have become essential to the socio-economic growth of developing countries. The Convention on Biological Diversity (CBD) has brought new awakening about the hitherto unknown/unrealized potential of biodiversity, its conservation and utilization in a manner that would ensure both natural evolution and sustainable development. (Pushpangadan, 2002; Anon., 2011). Uttarakhand state with a forest cover of about 64.8% is bestowed with a number of medicinal plants. These plants occupy an important position in the sociocultural, spiritual and medicinal arena of rural people of the State. Their sustainable utilization can be of immense benefits to the local and national economies and in particular offers a sustainable livelihood to the rural poor of the state. In spite of the enormous structural diversity of phytochemicals that has been revealed to date, so far only a small fraction of plants used in traditional system of medicines of the state has been assayed. There is a large number of plant species left uninvestigated from their chemical constituents point of view, and are considered of high potential for development of phytomedicines. There is, therefore, much current research devoted to the phytochemical investigation of the higher plants which have ethno botanical information associated with them. Prompted by above facts, chemical examination of the leaves of Pavetta indica (PI) and Scindapsus officinalis (SO) was conducted and the chemical constituents characterized in these plants are reported herein. These species were chosen on the basis of their documented use in traditional system of medicines, scanty reports on their chemistry, and occurrence in the Uttarakhand state.

Material and Methods

General experimental procedure

All the melting points are uncorrected. IR spectra in KBr were measured on a Jasco FT-IR spectrophotometer. All chemicals and reagents were of laboratory grade and purchased from standard commercial suppliers. The extracts were examined on TLC using adsorbents-silica gel and cellulose. Silica gel (100-200 mesh) was used for column chromatography and zones on TLC were detected using iodine vapors, 5% sulphuric acid, alcoholic $FeCl_3$ and Folin Reagent. Characterization of all the compounds was done by comparison with their authentic samples (m.mp., Co-tlc and suprimposable IR, except PIL4, PIL5 and SOL5) provided by Dr. Rameshwar Dayal, Ex-Head, Chemistry Division, FRI, Dehra Dun.

Plant material

The leaves of Pavetta indica and Scindapsus officinalis were collected from wild population of plants growing in adjoining areas of Dehra Dun. The plants were identified at the Systematic Botany Branch, Botany Division, FRI, Dehra Dun by comparison of our specimen with authentic specimen preserved in the herbarium of the institute.

Extraction and isolation of compounds from Pavetta indica leaves

Air dried and milled leaves (500 g) were extracted with hot petroleum ether (60-800C), chloroform, acetone and 25% aqueous methanol (2.5 l x3) for 6 h. Removal of the solvent under reduced pressure yielded their respective extracts. Examination of the petroleum ether extract on TLC did not reveal any prominent spot. Yield of the extract was also poor. It was, therefore, not analyzed further. Column chromatography of the chloroform extract over silica gel using chloroform as eluant followed by TLC examination of the eluted fractions afforded one pure compound as white crystals (PIL1). The acetone extract was concentrated to dryness to determine the yield but while repeating the experiments the acetone extract was concentrated to 200 ml and allowed to stand over night. A solid got separated which was filtered and washed with acetone to obtain a white solid. Column chromatography of the solid over silica gel using gradient elution with $CHCl_3$ - MeOH yielded one pure compound (PIL2). The aqueous methanol extract was sequentially extracted with ethylacetate and butanol, and respective extracts (PILEA and PILBU), after removal of the solvent under reduced pressure, were obtained. The PILEA extract was subjected to column chromatography over silica gel using successive elution with $CHCl_3$ -MeOH. The fractions were collected and examined on TLC. One pure compound as yellow needles (PIL3) was isolated. The PILBU extract did not show any prominent spot on TLC and hence was not analyzed further. The aqueous methanol extract was also hydrolyzed with 2M HCl for 0.5 h and extracted with diethyl ether. The ether extract was examined on 2D TLC (silica gel) using mobile phase 10% acetic acid in chloroform and 45% ethylacetate in benzene. A blue colored spot developed with Folin reagent after fuming with ammonia indicated the presence of a phenolic acid (PIL4). The aqueous methanol extract was further hydrolyzed with 2M NaOH under N_2 for 4 h followed by extraction with diethyl ether after acidification. The ether extract was examined on 2DTLC (Cellulose) using mobile phases benzene: acetic acid: water (6:7:3) and 15% acetic acid in water. A blue colored spot detected under UV light indicated the presence of another phenolic acid (PIL5).

Extraction and isolation of compounds from Scindapsus officinalis leaves

Air dried and milled leaves (500 g) were extracted with hot petroleum ether (60-800C), chloroform and 25% aqueous methanol (2.5 l x3) for 6 h. Removal of solvent under vacuum yielded their respective extracts. The petroleum ether extract was column chromatographed over silica gel. Elution with varying amount of ethyl acetate in petroleum ether afforded wax (the largest component) and a pure compound (SOL1). Column chromatography of chloroform extract over silica gel using $CHCl_3$ and $CHCl_3$ -MeOH as eluants followed by examination of the collected fractions on TLC yielded a pure compound (SOL2). The aqueous methanol extract was sequentially extracted with ethylacetate and butanol, and removal of the solvent under reduced pressure produced the respective extracts (SOLEA and SOLBU). The SOLEA extract was column chromatographed over silica gel using $CHCl_3$ and $CHCl_3$ - MeOH as eluants. The fractions were collected and examined on TLC. Two pure compounds (SOL3 and SOL4) were isolated. The SOLBU fraction was dissolved in 1% methanolic HCl and examined on cellulose plate using butanol: acetic acid: water (4:1:5) as mobile phase. A magenta color spot indicated the presence of an anthocyanidin (SOL5),

Results and Discussion

Extraction of the PI leaves with hot petroleum ether, chloroform, acetone and 25% aqueous methanol followed by removal of the solvent under vacuum yielded their respective extracts. Colum chromatography of chloroform, acetone, and PILEA extracts over silica gel afforded three pure compounds PIL1, PIL2 and PIL3, respectively. PIL1 was obtained as white crystals (m.p. 1880C) and responded a positive Liebermann- Burchard test for terpenoids and steroids. It was found identical with authentic sample of ?-amyrin. PIL2 obtained as white powder was crystallized with chloroform- methanol and characterized as ursolic acid (m.p. 291-292°C) by direct comparison with authentic sample. Contemporary scientific research has revealed that several pharmacological effects, such as, anti-tumor, hepatoprotective, anti-inflammatory (oral and topical), anti-ulcer, antimicrobial, anti-hyperlipidemic and antiviral, can be attributed to ursolic acid (Liu, 1995). The activity of ursolic acid compared well with the known hepatoprotective drug, silymarin (Shukla et al. 1992). PIL3 obtained as yellow needles (m.p. 236°C) gave green color with alcoholic $FeCl_3$ and positive Molisch test. This was found identical with an authentic sample of quercetin -3-O-galactoside. PIL4 and PIL5 appeared as blue colored spots on examination of the acid hydrolyzed and alkali hydrolyzed aqueous methanol extract on TLC (silica / cellulose) were identified as p-hydroxybenzoic acid and caffeic acid, respectively, by comparison with the authentic compounds. Both p-hydroxybenzoic acid and caffeic acid are important phenolic compounds with pharmaceutical importance (Sachan et al. 2006). Thus, five compounds belonging to

the group of triterpenes (?- amyrin and ursolic acid), flavonoids (quercetin-3-O-galactoside), and phenolic acids (p-hydroxybenzoic acid and caffeic acid) were characterized in the PI leaves (Figure 1). Identification of ursolic acid and quercetin -3-O-galactoside is the first report of their occurrence in the PI leaves.

The leaves of SO were extracted with hot petroleum ether, chloroform and 25% aqueous methanol and respective extracts were obtained after removal of the solvent under vacuum. These extracts when subjected to column chromatography over silica gel afforded five pure compounds SOL1 (from petroleum ether extract), SOL2 (from chloroform extract), SOL3, and SOL4 (from SOLEA extract). SOL1 was obtained as white silky needles (m.p.1370C) and characterized as ?- sitosterol by comparison with the authentic sample. SOL2 was crystallized from methanol as yellow needles (m.p. 316 0C). It gave green color with alcoholic FeCl3 and red color with Mg/ HCl. It was characterized as quercetin by comparison with the authentic sample. Quercetin is reported to be associated with several bioactivities such as antioxidant, anti-inflammatory, anti-neoplastic, antiplatelet and antiviral, etc. (Spoerke and Rouse 2003; Murray and Bongiorno, 2006). SOL3 obtained as off white needles (m.p. 222-230C) produced a dark spot on TLC when sprayed with dilute sulphuric acid and subsequently heated. It gave brownish color with alcoholic FeCl3. The compound was found identical with authentic dihydrokaempferol. SOL4 obtained as yellow needles (m.p. 191-1920C), after crystallization from a mixture of ethanol and water (1:1, v/v), gave green color with alcoholic FeCl3 and positive Molisch test. It was characterized as rutin (quercetin -3- rutinoside) by comparison with the reference standard. Rutin is a well known antioxidant and used in herbal medicines as a capillary / blood vessel protectant (Harborne et al. 1999; Murray and Bongiorno, 2006). SLO5 appeared as magenta colored spot on examination of the acid hydrolyzed butanol fraction on TLC (cellulose) was characterized as cyanidin by comparison with the authentic compound. Thus SO leaves were found to contain five constituents belonging to the group of flavonoids (two flavonols- quercetin, rutin; flavanonol-dihydrokaempferol; anthocyanidin- cyanidin), and triterpenes (?- sitosterol) (Figure 2). Occurrence of these compounds in the SO leaves is reported for the first time.

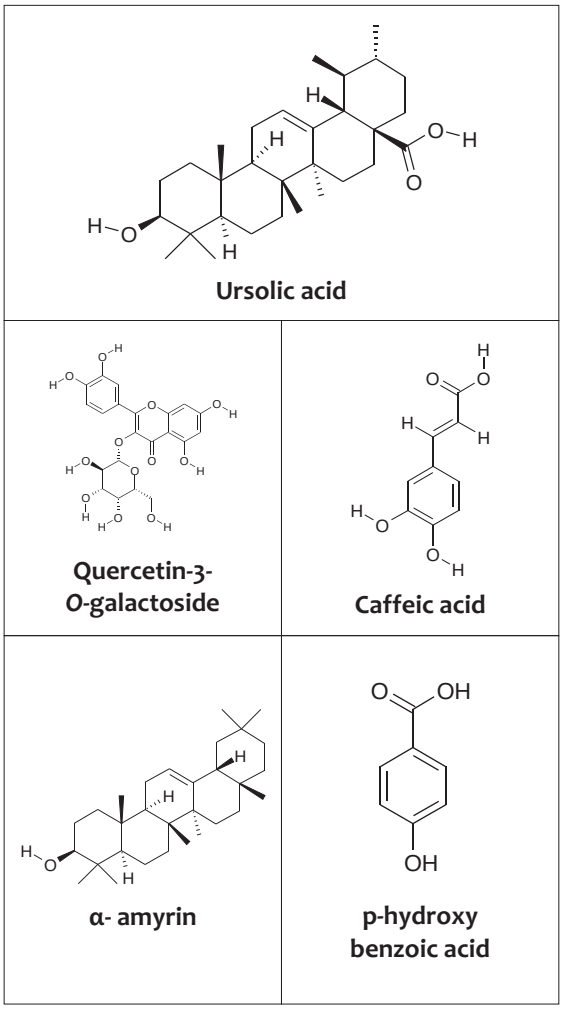
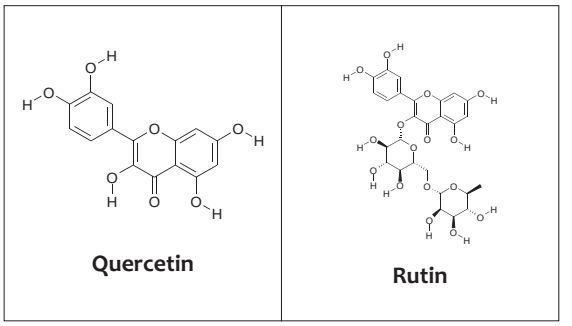


Fig.1: Chemical constituents characterized in leaves of Pavetta indica



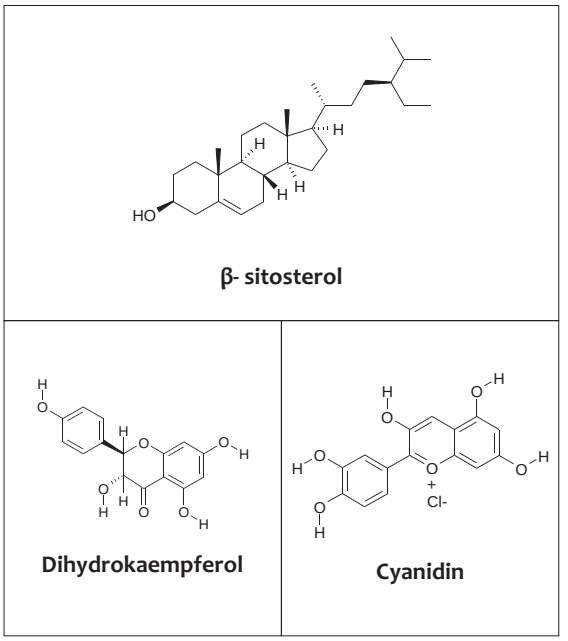


Fig.2: Chemical constituents identified in leaves of *Scindapsus officinalis*

Acknowledgement

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Antibiotic Effect Of Murraya Koenigii And Their Therapeutic Application As An Anticancer Herb

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Abstract - Plant derived drug serve as prototype to develop more effective and less toxic medicines. Murraya koenigii (Bengali name: Meetha neem, Family: Rutaceae) is an aromatic shrub. The plant has been used in folk remedies by Indians and is reported to have a broad range of therapeutic effects, including analgesic, anti-inflammatory, febrifuge activity and is useful in leucoderma and blood disorders.
The Leaves also relieve nausea, indigestion, vomiting; eaten as a cure for diarrhea and dysentery. There are also some reports confirmed its anticancer properties.
The purpose of the present study was to evaluate Murraya koenigii leaves as potential source of natural antibiotic. The studies conformed that the leaves was active against S. aureus and B. subtilis and other pathogenic bacteria. This study might be useful to supplement information in regard to its identification as potent antimicrobial agent.

Key words: Antimicrobial activity, natural antibiotic, Murraya koenigii

Introduction

In recent years, ethnobotanical and traditional uses of natural compounds, especially of plant origin received much attention as they are well tested for their efficacy and generally believed to be safe for human use. They obviously deserve scrutiny on modern scientific lines such as phytochemical investigation, biological evaluation on experimental animal models, toxicity studies, investigation of molecular mechanism of action (s) of isolated phytoprinciples and their clinical trials. It is a best classical approach in search of new lead molecules for management of various diseases.
Many plants are used as folk medicines to infectious diseases such as urinary tract infections, diarrhea, cutaneous abscesses, bronchitis and parasitic diseases have been known throughout the history of mankind.
Efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (Bandow JE et al,2003).
Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity (Heinrich M and Gibbons S,2001). India is one of the pioneers in the discovery of herbal medicines for the treatment of various ailments. In the ancient past, India exported many such drug plants to the oriental countries as well as to Greece, Italy, Egypt and Arab countries. With the development of Western therapy people lost interest in herbal medicines. In recent years this trend has reversed. Intensive research in this area is now being pursued all over the world. Serious efforts are being made by the phytochemists and botanists in exploring the plant world to discover more potent drugs from botanical species. These studies have culminated in the isolation, structure elucidation and emphasis is being given on herbal medicines because of their ready availability and minimal side effects. Crude drugs in many cases are found to be more potent than the pure drugs, the reason being due to the synergistic action of the other components present which not only enhance the biological activity of the drug but simultaneously lowers the toxic effect. Till now, the traditional herbal drugs remain the major source of health care for more than two thirds of the world's population. The World Health Organisation is strongly advocating for wider acceptance and use of traditional medicine including Ayurveda and Unani (Iwaki, K et al,2006). Essential oils, the vast reservoir of secondary metabolites produced by aromatic and officinal plants are of specific interest due to potent biological activities (Pattnaik S.et al,1997 &, Durate MCT et al,2007). They are complex mixture of monoterpenes and sesquiterpenes which are hydrocarbons with the general formula $(C_5H_8)_n$.
Murraya koenigii (Bengali name: Meetha neem, Family: Rutaceae) is an aromatic shrub or small tree found throughout India and mainly cultivated for its aromatic leaves. Leaves are used as a condiment in the preparation of curry powder, pickle, chutney, sausages and seasonings. Leaves relieve nausea, indigestion, vomiting; eaten as a cure for diarrhea and dysentery.
Cancer A study published by P. Muthumani, et al., in the "Journal of Pharmaceutical Sciences and Research" in 2009 showed a significant decrease in the cancer cell number and tumor weight of rats with Dalton's ascitic lymphoma after treatment with curry leaf extracts. The extract also showed significant anti-inflammatory activity in the rats. Chronic inflammation is a precursor to a number of diseases, including cancer,

atherosclerosis and Alzheimer's disease.

As a part of our continuing study on chemical and biological characterization of different plants, attempt was made this time to investigate the antibacterial activity of Murraya koenigii leaves against different Gram-positive and Gram-negative bacteria.

Materials And Methods

Plant Material

Leaves of Murraya koenigii Spreng. were collected from the local supplier in Dehradun in the month of September, 2012. The plant was identified and authenticated in Indian Forest Research Institute, Dehardun

Extract Preparation

The leaves were collected and washed thoroughly in water, chopped, air dried for a week at 35-40 °C and pulverized in electric grinder. 150 gm. of the powder subjected to soxhlet apparatus using solvents such as Hexane and Methanol. The solvent was then removed under reduced pressure, which obtained a greenish-black colored residue. The yield was 7.4% and 5.9% respectively. The prepared extracts were used for the antimicrobial activity.

Antimicrobial Study

Microorganisms

Three strains E. coli, Bacillus subtilis, Staphylococcus aureus, were used for assessing the antimicrobial activity of the plant extract and standard Ciprofloxacin. The microorganisms were obtained from the Institute of Microbial Technology (IMTECH) Chandigarh.

Study Protocol

Modified agar diffusion method of (Iwaki, K et al,2006)was adopted for this study. Molten agar medium (25 ml) was taken in a sterile petridish and broth cultures of the test isolate (0.1 ml) containing 1.0 X 105 CFU/ml of organism were added. The contents were thoroughly mixed and allowed to solidify. The extracts were dissolved in DMSO and used in 10, 20, 40 and 50 mg/ml concentrations. Ciprofloxacin was used as standard antibacterial and antifungal agents. Wells were bored in the plates, using a standard sterile cork borer of 8 mm diameter and equal volumes of the plant extracts was transferred into the well with using micropipette. The plates were kept for 1hr for pre-diffusion and incubated at 37°C/24hr. At the end of incubation, zone of inhibition was measured in all the plates.

Results And Discussion

Methanol extract exhibited prominent antibacterial activity (Table 1,2&3). The extract is showing good inhibitory properties for Staphylococcus aureus followed by Bacillus subtilis and *E.coli.*

The studies conformed that the methanolic extract of leaves was active against S. aureus and B. subtilis with maximum inhibition zone diameter (15mm) & (14mm) respectively and moderate effect against E.coli followed by water extract and no activity was shown by hexane extract.

An intense search of the literature has revealed that the stems, leaves, roots and seeds are potential sources of carbazole alkaloids of common and lesser known skeleta.

Table-1 Antibacterial activity against *Escherichia coli*

Leaf extracts	Zone of inhibition (mm) Diameter of bore is 6mm		
	Sample 50µl	Negative control 50µl	Positive control (Ciprofloxacin) 50µl
Water	11	No activity	28
Methanol	13	No activity	
Hexane	No activity	No activity	

Table-2 Antibacterial activity against *Staphylococcus aureus*

Leaf extracts	Zone of inhibition (mm) Diameter of bore is 6mm		
	Sample 50µl	Negative control 50µl	Positive control (Ciprofloxacin) 50µl
Water	12	No activity	29
Methanol	15	No activity	
Hexane	No activity	No activity	

Table-3 Antibacterial activity against *Bacillus subtilis*

Leaf extracts	Zone of inhibition (mm) Diameter of bore is 6mm		
	Sample 50µl	Negative control 50µl	Positive control (Ciprofloxacin) 50µl
Water	12	No activity	23
Methanol	14	No activity	
Hexane	No activity	No activity	

The present investigation revealed that the essential oil of M. koenigii leaves exhibited antimicrobial properties which explain the basis for its use in traditional medicines.

Thorough screening of literature available on Murraya koenigii depicted the fact that it is a popular remedy among the various ethnic groups, Vaidyas, Hakims and ayurvedic practitioners for cure of variety of ailments. Following the traditional and folk claims, very little efforts have been made by the researchers to explore the therapeutic potential of this plant. It is interesting to note that crude organic extracts of leaves of Murraya koenigii have been screened for some pharmacological activities and found to possess anti diabetic, cholesterol reducing property, anti diarrhea activity, cytotoxic activity antioxidant property, antiulcer activity antimicrobial, antibacterial potential.

Girinimbine, a carbazole isolated from the bark of Murrya koenigii significantly induced programmed cell death in HepG2 cells suggesting the necessity for further evaluations in preclinical human hepatocellular carcinoma models (Syam et al,2011).

Till other parts of plant such as seeds, leaves and seed oil which are documented to possess important medicinal virtues, are not explored scientifically for their biological potential. In future study, the isolated principles from curry leaf needs to be evaluated in scientific manner using scientific experimental animal models and clinical trials to understand the molecular mechanism of action, in search of lead molecule from natural resources.

Conclusion

The plant Murraya koenigii Spreng. (Rutaceae) has been used in folk remedies by Indians and is reported to have a broad range of therapeutic effects, including analgesic, anti-inflammatory, alexiteric,febrifuge activity and is useful in leucoderma and blood disorders.

The significant activity of M. koenigii against B. subtilis which is food contaminants makes it a promising antimicrobial for food preservation.

In vitro antimicrobial activity of leaf against common pathogens justifies the folk medicinal use of curry leaf tree for the treatment of diarrhea, dysentery and skin eruptions.

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Wound Healing Activity of Ursolic Acid Stearoyl Glucoside Isolated from *Lantana camara*

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Abstract - Leaves of Lantana camara L. is traditionally used for cuts, swellings and burns for their healing property. Present study designed to evaluate the wound healing activity of ursolic acid stearoyl glucoside (UASG) isolated from Lantana camara. UASG isolated from Lantana camara (100 mg/kg/day) was evaluated for its wound healing activity in albino rats using excision and incision wound models. UASG treated animals exhibited significant ($P<0.01$ and $P<0.001$) reduction in wound area when compared to controls. UASG treated wounds are found to epithelize faster as compared to controls. Significant ($p<0.01$) increase in granuloma breaking strength was observed when compared with control group. The wound healing activity of UASG from Lantana camara was studied by using excision and incision wound model and the UASG showed the significant wound healing activity.

Keywords: Lantana camara; ursolic acid stearoyl glucoside; wound healing

Introduction

Ursolic acid is one of the best known bioactive pentacyclic triterpenoids. It is widely found in more than 120 plant species; most of them used as medicinal plants in traditional medicine and also exist in food products (Price et al., 1987). Ursolic acid is of great interest to scientists because of its several biological activities. These include anti-inflammatory (Gupta et al., 1969), antiulcer (Gupta et al., 1981), cytotoxic (Lee et al., 1988), antiproliferative, antiviral (Yim et al., 2006), and antidiabetic effect (Kazmi et al., 2012). In view of its easy availability in many plants and strong traditional evidence of wound healing property of plant, we have carried out a evaluation of wound healing activity of ursolic acid stearoyl glucoside isolated from Lantana camara.

Ursolic acid stearoyl glucoside (UASG), pentacyclic triterpenoid isolated from Lantana camara L. (family: verbanaceae) (Kazmi et al., 2012). The traditional uses of Lantana camara L. mainly refer to the treatment of asthma, ulcers, measles, chickenpox, eczema, tumors, cancers, high blood pressure, bilious fevers, catarrhal infections, tetanus, rheumatism, malaria, ataxy of abdominal viscera [9,10], memory weakness, enhance intellect and cognition (Adams et al., 2007). The plant has reported as anticonvulsant (Adesina, 1982), anticancer (Bisi-Johnson et al., 2011;)[13, 14], antiulcer [15], antioxidant [14], anti-diabetic [16, 17], antifungal, antibacterial [18-20], anti-feedant, larval mortality/repellency [21-23], anti-motility [24], analgesic and anti-inflammatory [25] activities.

Hence, in the present study UASG isolated from Lantana camara L. was was evaluated for its wound healing activity in albino rats using excision and incision wound models.

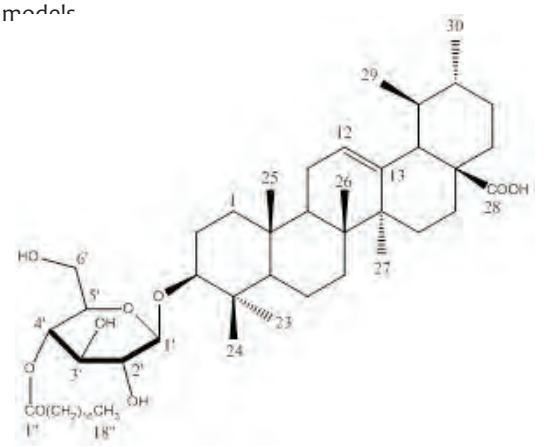

Fig.1- Structure of Ursolic acid stearoyl glucoside

Materials And Methods

All the studies conductedwere approved by the Institutional Animal Ethical Committee Siddhartha Institute of Pharmacy, Dehra Dun, India (1435/PO/a/11/CPCSEA).

Plant material

Lantana camara L. leaves were collected from Hamdard University and identified by Dr. S. B. Singh, Scientist, NISCAIR, New Delhi. A voucher specimen (NISCAIR/RHMD/consult/-20-09-10/1322/125) was deposited in the herbarium of NISCAIR, India.

Extraction and isolation of UASG

Dried powder of Lantana camara leaves (4 kg) was extracted with methanol (12 L) at 50 °C for 1 day. Extract was concentrated to dryness under reduced pressure to

obtain slurry (605 g). The slurry was dissolved in minimum amount of methanol and was adsorbed on silica gel (60-120 mesh). The slurry was subjected to a silica gel column using CHCl3/ MeOH gradient system (49:1; 2.0 L for gradient system); leads to elution of colorless crystals of USAG (yield 11.2 g, 0.28 %). Same experiment was repeated to collect more amount of UASG required for wound healing activity. It was found to be 100 % pure by HPTLC by using solvent system CHCl3/ MeOH (99:1).Structure of compound was identified by comparison of their spectroscopic data from the reported literature [1]. The structure of USAG is depicted in Fig.1.

Animals

Wistar albino rats (150-200 g) were obtained from Animal House, Siddhartha Institute of Pharmacy, Dehradun and kept at 25 1 C, 55 5 % humidity along with 12 hr light/dark cycle. The animals were given standard pellet diet (Lipton rat feed, Ltd., Pune) and water ad libitum throughout the experimental period. The experiment was approved by the 'Institutional Animal Ethics Committee'.

Chemicals

Framycin sulphate cream 1% w/w (Ranbaxy, India), diethyl ether, ethanol, sterilized cotton were purchased from Chopra Pharmaceuticals, New Delhi. All other chemicals used were of analytical grade. Ursolic acid stearoyl glucoside was suspended in Tween 80 in saline and used.

Wound healing activity

Excision and incision wound models were used to evaluate the wound-healing activity of USAG. Studies conducted were approved by the Institutional Animal Ethical Committee (1435/PO/a/11/CPCSEA) of Siddhartha Institute of Pharmacy, Dehradun, India.

Wistar albinorats of either sex weighing between 150-200gms were divided into six groups of six animals each.

Group I: Received no treatment and served as control.

Group-II - Received application standard framycin sulphate cream 1% w/w.

Group-III - Received application of UASG (100 mg/kg/day.).

Excision wound model

Excision wounds were used for the study of rate of contraction of wound and epithelization. Animals were anaesthetized with slight vapour inhalation of di-ethyl ether and the right side of each rat was shaved. Excision wounds sized 300 mm2 and 2 mm depth were made by cutting out layer of skin from the shaven area. The entire wound was left open. The treatment was done topically in all the cases. The UASG was applied at a dose of 100 mg/kg/day for 15 days. Wound areas were measured on days 1, 3, 6, 9, 12 and 15 for all groups, using a transparency sheet and a permanent marker (Nayak et al., 2007).

Incision wound model

The incision wound model was studied. Under light ether anesthesia the animal was secured to operation table in its natural position. One paravertebral straight incision of 6 cm was made on either side of the vertebral column with the help of scalpel blade. Wounds were cleaned with 70% alcohol soaked with cotton swabs. They were kept in separate cages. UASG was applied at a dose of 100 mg/kg/day for 10 days. The sutures were removed after 8 days, on tenth day the tensile strength was measured by continuous constant water supply technique (Baie and Sheikh, 2000).

Statistical Analysis

The means of wound area measurement and wound breaking strength between groups at different time intervals were compared using one-way ANOVA, followed by Tukey's tests.

Results And Discussion

UASG was administered to the test groups in graded doses ranging up to 100 mg/kg body weight/day and the rats were observed 10 days for any signs of mortality and behavioural disabilities. Then dose was increased upto 500 mg/kg and again observed for signs of mortality and behavioural disabilities for another 10 days. Its LD50 value was found to be higher than 500 mg/kg body-weight in rats. 1/10th and 1/20th of 500 mg/kg of UASG was used for the further experimentation.

In studies using excision wound model, the UASG treated group III showed significantly greater wound healing as compared to standard drug treated animals (Table-1). In incision wound model, significant increase

Table-1 - Effect of UASG on Excision Wound [Wound Area (mm2)]

Day	Group I	Group II	Group III
0	268.15±6.19	251.28±5.88	271.25±4.86
3	238.45±8.40c	198.16±4.36c	214.43±6.17b
6	210.18±8.01b	152.26±1.99c	159.26±2.19b
9	181.47±3.17c	111.33±1.78b	110.51±1.81c
12	133.49±2.27c	056.54±1.06b	043.35±0.92b
15	072.36±3.17b	017.58±0.13b	013.35±0.25c

All values are mean ± SEM; n=6.
b P<0.01, c P<0.001, when compared to control.

Table-2 - Effect of UASG on wound healing in incision wound

Groups	Incision wound breaking strength (g)
Group I	279.27±10.17
Group II	467.34±13.19b
Group III	471.40±15.45b

All values are mean ± SEM; n=6.
b P<0.01, c P<0.001, when compared to control.

In present study incision wounds healing by granulation, collagenation, and tensile strength was measured indirectly to assess the collagen content and maturation. The results indicate that UASG significantly promoted collagen as compared to that of control.

Use of single model is inadequate and there is no reference standard which can collectively represent the various components of wound healing as drugs which, influence one phase may not necessarily influence another. Hence in our study we have used two models to assess the effect of UASG on various phases of wound healing.

Conclusion

The wound healing activity of UASG from Lantana camara was studied by using excision and incision wound model and the UASG showed the significant wound healing activity as

like as standard FSC (Framycetin sulphate cream).

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Quantitative Analysis of Curcumin in Dosage Forms Using H.P.L.C.

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Abstract - Curcumin quantification in dosage forms using high performance liquid chromatography (HPLC) has been carried out. This experiment was aimed to determine analysis conditions to be applied in curcumin dosage form analysis using HPLC. The experiment has been done with the following steps: mobile phase preparation, instrument preparation, working solution preparation followed by precision assay, accuracy assay, standard curve estimation, and sample measurement. chosen condition was HPLC, LC-10AT, Shimadzu; column Shimadzu C18, VP-ODS, acetonitrile-2% as mobile phase, acetonitrile as solvent; detection at 420 nm; flow rate 1.2 mL/min. The result showed a correlation coefficient value was 0.999, recovery value for accuracy assessment was 99.50%, variation coefficient for precision was 1.33%, limit of detection was 0.7816 ppm, and limit of quantification was 2.6053 ppm. Thus, the analytical method using HPLC for curcumin were feasible for curcumin quantification in dosage forms. This method has been applied for measuring three curcumin dosage forms A, B, C, and one curcumin raw material D. Samples A, B, C, and D measurement gave 105.86, 87.12, 10.71, and 130.35%, respectively.

Introduction

Curcumin is a principal curcuminoid of spice turmeric. Curcumin is an orange yellow coloring principle obtained by solvent extraction of turmeric. Pharmacology activities for curcumin are antibacterial, antifungi, antihepatotoxic, analgesic, antiinflammation, anticholesterol, choleretic, antioxidand, and so on. Cholagogum activity is marked by increase of bile production and secretion that worked choleretic and cholekinetic (Sidik 1992). By increasing bile secretion, it will decrease solid particles in gallbladder. This condition reduces bile cholic, stomach puffing caused by fat metabolism disorder, and lower blood cholesterol level (Dalimartha 2001). Curcumin and xanthorrizol are marker compounds in curcuma rhizomes (Badan POM 2004). Research of dosage form with herbal drugs as raw material nowadays focused on isolation, identification, and pharmacological study of active substance, while quantitative analysis of active substance in herbal drug that might be unstable after distribution is rarely found. Curcumin quantitative analysis in 1983 were carried by high performance liquid chromatography (HPLC) method using Nucleosil-NH_2 column and reversed phase with ethanol as mobile phase, and fluorometer detector. Some curcuminoid analytical methods using HPLC such as mix of acetonitrile:acetic acid 5% (51:49) as mobile phase and ultraviolet detector, trifluoroasetic acid:water (40:60) and visible detector, acetonitrile:acetic acid 0,25% and visible detector, and acetonitrile:acetic acid 7,6% (55:45) with visible detector. The relative polar property of curcuminoid and consisting hydroxyl chromophore group makes its quantitative analysis able to be carried out by using reversed phase system with octadesilsilane column and ultraviolet-visible detector.

Material And Method

Material

The materials used were curcuminoid and acetonitrile.

Equipment

HPLC, LC-10AT, Column: C18, VP-ODS 150L, Membrane filter 0.45 ?m, Syringe filter 0.45 ?m.

Determination of Analysis Condition

Mobile phase preparation

1. An amount of 20 mL glacial acetic acid was measured and then put into 1 L beaker glass; 980 mL aqua bidestilata was added. The mixture was stirred using magnetic stirrer for 10 minutes to obtain homogeneous solution. The solution was then filtered by pump filter; the filtrate was placed in Erlenmeyer flask and degassed. The solution was then placed into mobile phase bottle and labeled FG-Ac 2%.

2. An amount of 1 L acetonitrile was placed in beaker glass. The solution was filtered by pump filter, then placed in mobile phase bottle and labeled FG-ACN.

Instrument Optimation

HPLC column was cleaned up by elution, filtration, and degasion. Elution runs for 1 hour, then the column was washed using acetonitrile for 1 hour. After washing step, the column was conditioned by eluting mobile phase 2% acetic acid and acetonitrile (45:55) for 30 minutes and at the same time it was run for baseline.

Working solution preparation

For standard solution 100 ppm, 50 mg curcuminoid standard was weighed accurately, diluted in 50 mL volumetric flask upto the limit mark with acetonitrile, then 2.5 mL was taken and diluted into 25 mL volumetric flask with the same solvent. Procedures for other standard concentrations were done similarly.

Linearity and Range Study

Six concentrations of curcuminoid standard solution were made: 5, 10, 20, 40, 80, and 100 ppm. Each solution was filtered by syringe filter 0.45 ?m. The solutions were injected into injector once for each time and the area under curve was recorded and measured for the r2 and r values.

Presicion Study

Ten solutions of 20 ppm curcuminoid were made for standard solutions. Each solution was filtered by syringe filter 0.45 ?m. The solutions were injected into injector once for each time and then the area under curve was recorded and measured for the coefficient variation values

Accuracy Study

Three concentrations of curcuminoid standard solution were prepared for 20, 40, and 80 ppm. Each solution was filtered by syringe filter 0.45 ?m. The solutions were injected into injector three times for each silution and then area under curve was recorded and measured for the average recovery values.

Limit of Detection and Limit of Quantification Study

Six concentrations of curcuminoid standard solution were prepared: 5, 10, 20, 40, 80, and 100. Each solution was filtered by syringe filter 0.45 ?m. The solutions were injected once for each time and then the area under curve was recorded and measured for the standard deviations, limit of deviation (LOD), and limit of quantitation (LOQ) values.

Standard curve estimation

Six concentrations of curcuminoid sandard solution were prepared: 5, 10, 20, 40, 80, and 100 ppm. The solutions were injected into injector once for each and the area under curve was recorded and measured for the correlation coefficient in linear regression equation ($Y = ax + b$).

Quantitative Analysis

Sample solution preparation

Dosage form sample. Two samples of tablet were weighed and powdered to homogeneous. The powder sample was weighed for 1 dosage and diluted into 25 mL volumetric flask; 5 mL solution was taken and diluted with the same solvent into 10 mL in a volumetric flask. The solutionwas filtered with 0.45 ?m Whatman paper, and placed into HPLC vials. For capsule dosage form, similar steps were carried out as for tablet, with adjusted dilution for curcuminoid concentration in sample.

Curcuminoid raw material sample. Fifty mg curcuminoid sample was weighed accurately, diluted in 50 mL volumetric flask with acetonitrile, and then 2.5 mL of the solution was taken and diluted into 25 mL volumetric measure flask with the same solvent. The solution was filtered with0.45 ?m Whatman paper, and placed into HPLC vials.

Concentration measurement

Eighty ppm solution from the stock solution of four samples was injected into injector twice for each sample. The area under curves were recorded and plotted into standard curve linear regression equation.

Results And Discussion

The chosen experiment condition obtained for HPLC was using mobile phase consisted of acetic acid 2% and acetonitrile 55:45. The chosen solvent was acetonitrile. The curcumin linear regression curve was y = 36300x - 2771.6, with correlation coefficient r = 0.9999. Recovery results at nine concentrations measured in this experiment gave 99.50%. The presicion study resulted from ten concentrations measured in this experiment gave variation coefficient of 1.33%. LOD and LOQ study gave 0.7816 ppm and 2.6053 ppm, respectively.

Sample Quantitative Analysis

Quantitative analysis of three dosages form samples and one curcuminoid raw material samples were as foll ows (given in Fig.1) average concentration of A - 105.86%, B- 87.12%, C- 10.71% and D- 130.35%.

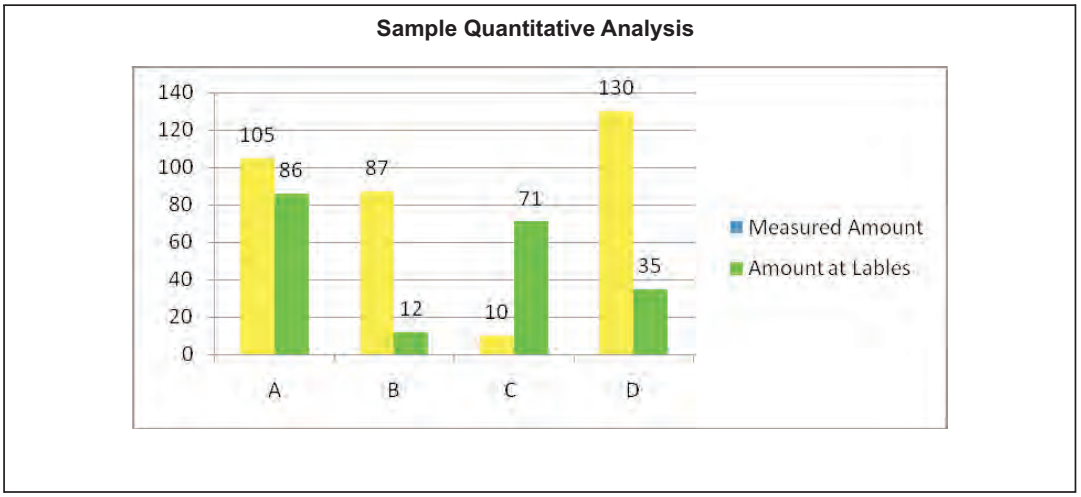

Fig.1 - Results of Sample Quantitative Analysis-

The method used in this experiment refers to Yang et al. (2006), however some conditions such as mobile phase, flow rate, and injection volume were not applied to this experiment. They carried out curcumin analysis in Gan Zhi Ping capsules using Zorbax C18 column, mobile phase acetonitrile-7,6% acetic acid (55:45), flow rate 1mL/minute and injection volume 10?L, while the detection run at 420 nm. We conclude that the use of this method does not guarantee a good accuracy and precision when applied at different place and condition. Therefore, it is necessary to run an analytical validation method. Analytical validation method is an observation on certain parameters based on laboratory experiment to prove that those parameters meet their requirements. However, a valid method in a certain condition is not a guarantee to be valid in other conditions. The mobile phase used in this experiment was mixture of acetonitrile and 2% acetic acid. This is result of several trials until we got the best condition at 2% acetic acid for composition (45:55) with acetonitrile. Curcumin was observed as the last peak eluted. Tonnesen and Karlsen (1983) reported that curcuminoid from turmeric eluted at the first peak was bidesmethoxycurcumin, followed by desmethoxycurcumin and curcumin. The method validation from the chosen conditionswas started with standard curve estimation. The standard curve made from six concentrations of standard solutions at the range of 5 to 100 ppm gave a correlation coefficient (r) 0.9999. This value is taken from the third peak of chromatogram which was assumed as curcumin, as observed in the previous experiment and based on the largest area under curve considering curcumin as major compound of curcuminoid. Accuracy was estimated by recovery study using direct comparation with the standard. The used three different concentrations of standard solutions, which is 20, 40, and 80 ppm, with three replications for each measurement. The recovery value was 99.50%. This value meets the requirement for accuracy assay, which must be at the range of 98-102%. Presicion was estimated by measuring ten standard solutions with the same concentration (20 ppm). From ten solutions measured, the coefficient value was 1.33%. This value also meets acceptance criteria for precision assay, which must be below 2%.

Curcumin Quantitative Analysis in Samples

Samples in this experiment were three curcuminoid dosage form samples and one curcuminoid raw material sample. Sample A, B, and C are the dosage form samples, while sample D is raw material sample. This raw material estimation may be useful for small industry that does not own analytical method for curcumin quantitative analysis. Each samples measured twice. Sample A measurement gave curcumin concentration 105.86% from its real value on the label (100%). Curcumin concentration in sample B was 87.12%. It might be caused by inaccurate production or curcmin degradation during distribution and storage. However, the incorret value still meets the requirement range. For sample C, the result was only 10.71% curcumin. This value is far away from its real value on the label and did not meet the requirements. The deviation from the true value may cause a different indication. Sample D showed curcumin concentration of 130.35% from the label. This value is far above its true concentration on the label, which was 80%. It might be caused by improper isolation procedure and quantitative analysis on label.

Conclusions

From the results above, we can conclude that curcumin can be measured by HPLC reversed phase system, using C18 column; acetonitrile-2% acetic acid as mobile phase in a ratio of 45:55 and acetonitrile as solvent. Accuracy and precision is fulfilled by 99.50% for recovery, and variation coefficient for presicion is 1.33%. Whereas the limit of detection was 0.7816 ppm, and limit of quantification was 2.6053 ppm. With this analytical method, curcumin concentration in samples can be determined for samples A, B, C, and D (from the concentrations written in the label) as 105.86, 87.12, 10.71, and 130.35%, respectively.

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About Curry leaves (*Murraya koenigii*) on Front Cover

Botanical Name: Murraya koenigii

Hindi : Kathnim, Mitha neem, Curry or kurry patta, Gandhela, Bareanga
Bengali : Barsanga, Kariphulli
Gujarati : Goranimb, Kadhilimbdo
Kannada : Karibevu
Malayalam: Karriveppilei
Marathi : Karhinimb, Poospala, Gandla, Jhirang
Oriya : Barsan, Basango, Bhuraunga
Punjabi : Curry patta
Sanskrit : Krishna nimba
Tamil : Karivempu, Karuveppilei
Telugu : Karepaku

Pharmacognosy

Origin and Distribution: Curry leaf is found almost throughout India up to an altitude of 1500 mtrs. It is much cultivated for its aromatic leaves.

Macroscopic Features: The macroscopical studies revealed the shape of leaves of Murraya koenigii (L.) Spreng as obliquely ovate or somewhat rhomboid with acuminate obtuse or acute apex, bipinnately compound with exstipulate in alternate arrangement. The petioles were of 20 to 30 cm in length. The leaf had reticulate venation and dentate margin with asymmetrical base.

Microscopic features: Anomocytic stomata were found distributed on abaxial surface while the adaxial surface was without stomata. The Uniseriate multicellular trichomes were observed on both surfaces, more frequent on upper surface of midrib portion.

Medicinal properties: The leaf is used as a natural flavoring agent in various curries. Volatile oil is used as a fixative for soap perfume. The leaves, bark and root of the plant are used in the indigenous medicine as a tonic, stimulant, carminative and stomachic.
It also posses Anti-oxidant, Anti-diabetic, cholesterol reducing, antiulcer and antimicrobial activity.

Forthcoming Events

5th International Conference on Drug Discovery and Therapy

Starts: Monday, February 18th 2013 at 12:00 am
Ends: Thursday, February 21st 2013 at 12:00 am
Dubai, UAE

Plant Diseases and Resistance Mechanisms

Starts: Wednesday, February 20th 2013 at 12:00 am
Ends: Friday, February 22nd 2013 at 12:00 am
Vienna (Austria)

3rd International Conference on Bioscience, Biochemistry and Bioinformatics - ICBBB 2013 Conference

24th to 25th February 2013
Rome, Italy
Website: http://www.icbbb.org/

All ICBBB 2013 papers will be published in the International Journal of Bioscience, Biochemistry and Bioinformatics (ISSN: 2010-3638), and all papers will be indexed by EBSCO, Google Scholar, and sent to be reviewed by Ei Compendex and ISI Proceeding
Organized by: CBEES
The conference aims to bring together scientists from industry, universities and research institutes to discuss modern academic and industrial aspects of various fields of development in science and technology of materials.
Organized by: Mansoura University - faculty of Science - Chemistry Department

11 th International Conference of Chemistry & its Role in Development Conference

11th to 14th March 2013
Mansoura & Sharm - ElSheikh, Dakahlia, Egypt
Website: http://www.mans.edu.eg

3rd International Conference on Chemistry and Chemical Process (ICCCP 2013) Conference

21st to 22nd April 2013
Beijing, United States of America
Website: http://www.icccp.org/

ICCCP 2013 will be published in IJCEA(ISSN:2010-0221), and all papers will be included in the E&T Digital Library, and indexed by EBSCO, WorldCat, Google Scholar, Cross ref and sent to be reviewed by Ei Compendex and ISI Proceedings.
Organized by: CBEES
Deadline for abstracts/proposals: 5th January 2013

1st Journal Conference on Bioscience, Biochemistry and Bioinformatics (JCBBB 2013 1st) Conference

21st to 22nd April 2013
Beijing, China

Website: http://www.ijbbb.org/jcbbb/1st/

All the registered papers will be published into IJBBB, ISSN: 2010-3638 by IACSIT Publishing, and distributed at the conference. The journal will be indexed by Google Scholar, Crossref, and Engineering & Technology Digital Library.

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June 2013
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Starts: Sunday, July 7th 2013 at 12:00 am
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61st International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Products Research (GA)

Starts: Sunday, September 1st 2013 at 12:00 am
Ends: Thursday, September 5th 2013 at 12:00 am
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COUNCIL INITIATIVES FOR PROMOTING HERBAL RESEARCH IN UTTARAKHAND

Uttarakhand State Council for Science and Technology (UCOST) was established in last quarter of the 2005 in Dehradun. Since its inception Council has encouraged the Research and Development activation in the State and has funded projects in various disciplines of Science and Technology. Council has provided financial assistance to peer reviewed projects and provide encouragement for researchers, scientists and for the development of trained manpower. Today the work initiated in 2005 has resulted in publications in reputed journals. I take pride in writing the references of the papers in herbal research that I have received from few of principal investigator of UCOST funded projects.

Aggarwal P et.al. (2009). Int. J. Chem. Sci., 7 (3): 1850; Univ. J. of Phyto-Chemistry and Ayurvedic Heights, 2:13-16; Mishra J et.al. (201 0). Int. J. of Intgr. Biol., 9 (1) : 26; Singh R. D. et.al. (201 0). India, Int. J. of Biol. and Biochem., 6(6):901 910; Sharma V et.al. (201 0). Asian J. of Microsoft Biotech Env. Sc., 12(3):677-680; Srivastava N et.al. (2010). J. of Plant Sci., 5(4):41 4-41 9; Int. J. of Green Pharm., 4(4):220-228; Singh P et.al. (201 0). Biol. I Elem. Res., 1 38:293-299; Negi J. S. et.al. (2009). Biol. I Elem. Res., 1 33:350-356; 1 35;27-282; Pharm. Rev., 4(8):21 5-220; (2010). Biol. T. Elem. Res., 136:364-371; 138:300-306: Nat. Prod. Comm., 5(6):907-91 0; (201 1). J. of Med. PI. Res., 5(1 0):1 900-1 904; Joshi S et.al. (2009). J. ofPI.Chromat.,22(6):l-3.

Council is also working in close association with Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknowfor its research endeavour in herbal plants. We also work in areas of science popularization and providing travel assistance to promote science and technology initiatives in the state. Many extension programmes are also executed by Technology Resource Centre (TRCs) located in the various part of the State. These impart training for cultivation and harvesting of herbal plants. In future Council intends to initiate many more R&D activities to improve the herbal sector of the State.

I extend my best wishes to Universities' Journal of Phytochemistry and Ayurvedic Height for their endeavour in Herbal research.

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