

NSHM Journal of Pharmacy and Healthcare Management

The official Publication of NSHM College of Pharmaceutical Technology

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Message from Chief Mentor

Over the last two decades the Indian Pharmaceutical sector has undergone a phenomenal change in almost all directions. In the current scenario it appears that to survive in this changing environment the Pharmacy community of India has to shift from its traditionally trodden path and become actively engaged in research, patent and publication. In this connection NSHM College of Pharmaceutical Technology (NCPT) has made its modest contribution by bringing out a yearly publication of NSHM Journal of Pharmacy and Healthcare Management.

In the present global scenario where meteoric advancements have taken place in the field of Pharmaceutical Research the need of the hour is to consolidate the expertise & the efforts made by the teachers, researchers & co-professionals engaged in this sector to provide a better health care and disease control regime for mankind.

As head of this institution, I take this opportunity to extend my whole hearted enthusiasm and best wishes to the Principal and all the Faculty members for their commendable effort for publishing fifth issue of “NSHM Journal of Pharmacy & Healthcare Management”.

Mr. Cecil Antony

Chief Mentor

NSHM Knowledge Campus

Message from the Founding Director

It is my great pleasure to write on the 5th volume of NSHM Journal of Pharmacy and Healthcare Management. Publication of the journal is the benchmark of academic excellence of any Institute of repute. The scientific journal is the collection of research works and reviews. My best wishes to the faculty members who worked with sincerity to bring forth this challenge.

I believe that this journal will fulfill the much needed platform for future researchers, academicians to share their views on various areas of research and technology. I wish the current editorial team all success.

Thanks & Regards,

Rajib Chanda

Co-founder & Director

Message from the Director

The ever growing and synergetic blend of technology and innovation is fuelling dynamism in global arena. There are a lot of challenges which the current scenario face the realms of basic necessities in life. Technology can play a very distinct role in bringing about this change. In the current scenario it appears that to survive in this changing environment the Pharmacy community of India has to change its old path and become actively engaged in research, patent and publication. In this connection NSHM College of Pharmaceutical Technology (NCPT) has made its modest contribution by bringing out 5th volume of NSHM Journal of Pharmacy and Healthcare Management.

On behalf of the entire NSHM team, I wish all the authors and reviewers who have submitted papers and/or provided valuable service as a reviewer for NSHM Journal of Pharmacy & Healthcare Management.

With best wishes,

Prof. (Dr.) Subhasis Maity

Director

NSHM College of Pharmaceutical Technology

NSHM Knowledge campus, Kolkata- Group of Institutions

Message from the Desk of Chief Editor

This is the fifth consecutive year that NSHM College of Pharmaceutical Technology is publishing its annual journal NSHM Journal of Pharmacy and Healthcare Management (NJPHM).

Since the inception of this college it has always been our earnest desire to motivate our students in pursuing higher studies and research for achieving a successful career in pharmacy. The publication of this annual journal is a humble approach in setting the pharmacy community on a course to a bright future.

I would like to thank the editorial team for their hard work and time spent in bringing the 5th volume of the journal.

With best wishes,

On behalf of Editorial Team

Prof. (Dr.) Tapan Kumar Barman

Principal

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Preliminary Phytochemical and Pharmacognostic study of *Crotalaria Spectabilis* Leaves

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Abstract

Crotalaria spectabilis Roth. is an annual woody herbaceous plant of Fabaceae and commonly known as Showy rattle box in English, Ghantarava in Sanskrit, Jhunjhun in Hindi & Pipuli jhunjhun in Bengali. The present study was aimed at preliminary pharmacognostic and phytochemical analysis of *C. spectabilis* leaves. Preliminary pharmacognostic study and physicochemical analysis was carried out according to the standard protocols to find out the distinct anatomical characters and the presence of different phyto-constituents in different solvent extracts of the leaves. Macroscopical study showed that the leaves were oblong, wedge shaped base. The length of the leaf lamina was found to be 13.9-14.8 cm and the breadth was 7-7.2 cm; length of the petiole was 0.8-1 cm. The upper surface was smooth, glabrous and the lower surface was slightly rough. Microscopical study explored the features like- vascular bundle surrounded by lignified pericycle, anomocytic stomata, presence of cystoliths etc. The total ash value was found to be 7.99% w/w. Phyto-chemical screenings showed the presence of steroid, terpenoid, glycosides, tannins, phenolic compounds and carbohydrates. The results may be useful to get desired therapeutic activity by identifying the standard variety and further to build up a monograph of the plant.

Key words: *Crotalaria spectabilis* Roth, leaves, pharmacognosy, physicochemical, phytochemical.

INTRODUCTION

The herbal medicines since from the Vedic lore captured a vast area in the management of several diseases.

Hence it is become very necessary to authenticate scientifically the particular species to yield desired therapeutic value from that. Pharmacognostic study is the preliminary step in the standardization of crude drugs. The detailed pharmacognostic evaluation gives valuable information regarding the distinct morphological and microscopic features of the plant species. Physicochemical analysis is an important parameter to standardize the variety. In the present study, preliminary pharmacognostic

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evaluation, physicochemical as well as phytochemical analysis of *Crotalaria spectabilis* Roth. had been performed to authenticate the variety.

C. spectabilis belonging to the family Fabaceae is an annual woody herb native to the Indo-Malaysia area. It has been planted widely & has naturalized in many tropical countries including the Southern United States, Hawaii & Puerto Rico. It is 1-2m tall [1]. It sports many branches covered with long velvety hairs. Oblong leaves are 4-9cm long with a wedge-shaped base. When mature, seed may break free inside the pods and create a rattling sound when shaken; hence the name is “rattlebox”. It serves as a nurse species during early reforestation and helps to protect the soil. It contains toxic pyrrolizidine alkaloids [2] (principally monocrotaline) which make it poisonous to livestock, particularly when seeds are consumed. Symptoms include photosensitization and liver disease within a few days to six months following consumption. In herbal medicine, extracts of the whole plant are used to treat impetigo and scabies, as an antiseptic for cuts and to treat intestinal worms [3-5].

No preliminary pharmacognostic & phytochemical evaluation had been reported for the leaves of this plant [6]. Therefore, the main aim of the present study was to perform the preliminary pharmacognostic investigation and physicochemical analysis of the leaves of *Crotalaria spectabilis* Roth. which would be further useful to authenticate the species or prepare a monograph for the proper identification of the plant.

MATERIALS AND METHODS

Collection and authentication: The mature leaves of *Crotalaria spectabilis* Roth.

(Fabaceae) were collected during January 2013 from Halisahar region of 24 Parganas district of West Bengal, India. The plant material was taxonomically identified at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India. Few samples of leaves were separated for the pharmacognostic study. The rest of the leaves were washed and then shade-dried with occasional shifting and then powdered with mechanical grinder, passing through sieve no. 40# and stored in an air-tight container for further physicochemical analysis.

Pharmacognostic Studies: [7]

- **Macroscopy-** Macroscopic characters of the leaves were studied such as size, shape, texture etc. Organoleptic study like color, odour, taste and texture of the leaf powder were also studied.
- **Microscopy-** Microscopic studies were performed by peeling off the upper epidermis and lower epidermis sections; free hand sections of midrib of leaves were also done and studied under microscope.
- **Histochemical tests-** Histochemical tests were performed under microscope with specific reagents to identify the specific histological characters.

Physico-chemical analysis:

Preliminarily physicochemical analysis of the powder drugs was performed as per the guidelines of Ayurvedic Pharmacopoeia of India. The testing parameters such as total ash value, extractive value etc. was performed [8, 9]. Nature of chemical constituents within the sample was broadly determined by performing qualitative

phytochemical tests of pet ether, chloroform and ethanol extracts of the leaves [10].

RESULTS AND DISCUSSIONS:

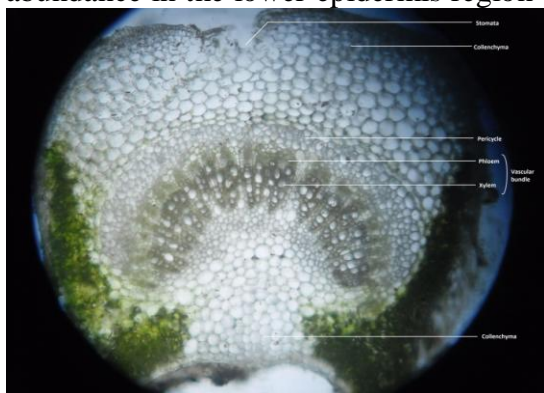
Macroscopic Characters:

Macroscopic study explored the shape of the leaf was oblong; leaf base was wedge-



Upper epidermis of leaf slightly rough.

A. Microscopic Characters: The detailed study of the transverse section of the leaf revealed the following microscopic character like the vascular bundle was surrounded by lignified pericycle, anomocytic stomata was present in abundance in the lower epidermis region



Transverse section of midrib shows stomata, collenchyma, pericycle and vascular wasbundle (having both xylem and phloem)

shaped. The length of the leaf lamina was 13.9-14.8 cm and the breadth of the leaf lamina was 7-7.2cm. The length of the petiole is 0.8-1cm. The upper surface was smooth and the lower surface was

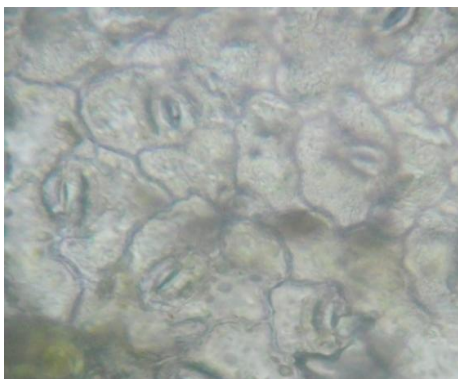


Lower epidermis of leaf

mainly, presence of cystoliths were found in the ground tissue, ca-oxalate crystals were also found at the base of trichomes, bunch of lignified spiral vessel and tracheids were also found in the ground tissue region.



Transverse section of midrib stained with Phloroglucinol-HCl, the vascular bundle surrounded by lignified pericycle



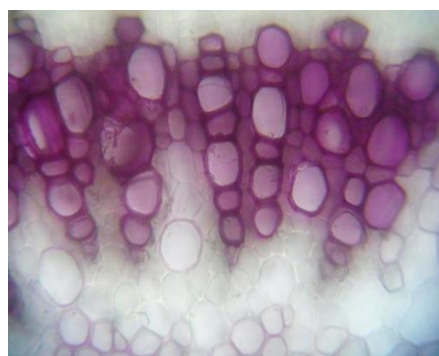
Anomocytic Stomata



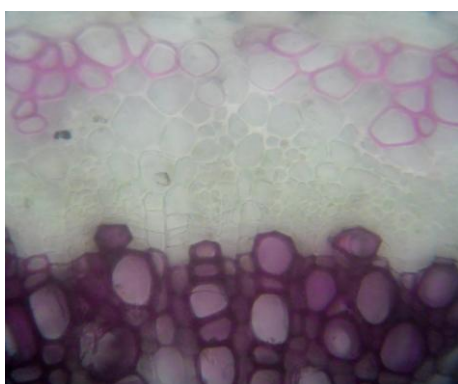
Crystal at the base of trichomes



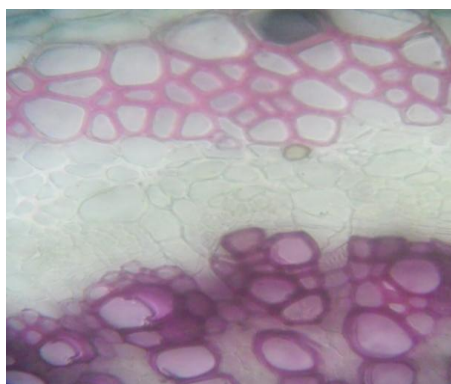
Calcium oxalate crystals



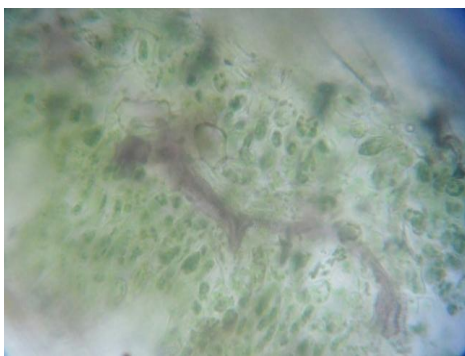
Clear view of stained Xylem



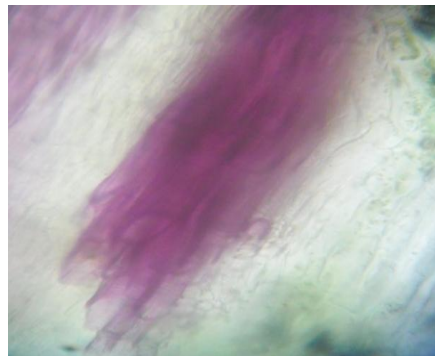
Phloem arranged between xylem and pericycle ring



Cystoliths



Lignified tracheid



Bunch of lignified trachea

B. Organoleptic Characters of Powdered Drugs (Leaf):-

Organoleptic study showed the powdered drugs were light green in colour, having astringent to bitter in taste with a distinct odour. The results are summarized in table 1.

Table 1: Organoleptic characters of the leaves of *C. spectabilis*:

Parameters	Inference
Colour	Light green
Odour	Distinct
Taste	Astringent, Bitter
Touch	Smooth
Texture	Amorphous

C. Histo-chemical tests: [10]

Histo-chemical studies were performed under microscope on the transverse sections of the leaves. Chemical reagents were used to identify the histological characters which assured the presence of lignin and starch in the sample.

Table-1

Results of Histo-chemical tests of the sample-Leaf of Ghantarava:

Sr. No.	Tests	Results
1.	Test for Lignin	+
2.	Test for Starch	+
3.	Test for Fixed oil	-

+ = Positive, - = Negative

D. Physicochemical Analysis:-

Among the physicochemical parameters the total ash value, extractive values etc. were performed following official procedures and the test results were noted down. The methanol soluble extractive value showed the highest value than the other two. All the results are composed in table 2.

Table-2: Analytical Values for Physicochemical parameters for the leaf sample of *Crotalaria spectabilis* Roth.

Sr.No.	Parameters (% w/w)	Sample
1.	Total Ash value	7.99%
2.	Alcohol Soluble Extractive	32%
3.	Chloroform Soluble Extractive	4%
4.	Ether Soluble Extractive	2%

Phyto-chemical Screenings:-

After performing the phytochemical screenings it was found that the ethanol extract was enriched with terpenoids, glycosides, tannins, phenols and carbohydrates. The pet ether extract showed the presence of steroids in it. All the results are summarized in table no 3.

Table-3: Results of qualitative tests of the sample-*Crotalaria spectabilis* Roth.

No.	Parameters	Pet ether extract	Chloroform extract	Ethanol extract
1.	Alkaloid	-	-	-
2.	Steroids	+	-	-
3.	Terpenoid	+	+	+
4.	Glycosides	-	-	+
5.	Tannins	-	+	+
6.	Proteins	-	-	-
7.	Phenolic compounds	-	-	+
8.	Carbohydrate	-	-	+
9.	Saponins	-	-	-
10.	Fixed Oil	-	-	-
11.	pH	5.5	6.5	6

+ = Positive, - = Negative

Conclusion:- The present study can be concluded under three categories

1. Review of Literature- *Shanapushpi* was interpreted by Chakrapanidutta

as *Ghantarava* which indicate sound producing quality of its dry fruits.

2. **Pharmacognostic Study-** In the macroscopic characters the shape of the leaf was oblong & the leaf base was wedge shaped. In the microscopic characters T.S of midrib showed stomata, collenchymas, pericycle and vascular bundle (having both xylem & phloem).
3. **Phytochemical Study-** The total ash value was 7.99% indicating presence of inorganic content in it. The alcohol soluble extractive value (32%w/w) was comparatively higher than the chloroform soluble extractive value (4%w/w) and ether soluble extractive value (2%w/w). The average pH value of the samples was 6.

The Pet ether and chloroform extract showed the presence of steroid and terpenoid in very intense amount and ethanol extract showed the presence of terpenoid, glycosides, tannins, phenolic compounds and carbohydrates. The chloroform extract showed a little higher pH than the other two extracts which may be for the solvent effect. The present study was aimed to establish the preliminary phytochemical & pharmacognostic characteristics of *Crotalaria spectabilis* (Roth) leaves. The results and observations found in the present study revealed the distinct pharmacognostic and physicochemical characters of the leaf of *Ghantarava* (*Crotalaria spectabilis* Roth.) which will help to prepare a proper guideline for the identification of this plant. It will also help to develop further improvements on standardization and classification of this particular variety in future [11,12].

Acknowledgement: The authors are very thankful to Dr. (Prof.) J. N. Pande, Principal, BIPS and Mr. Subir Pal, President, BIPS for their financial support and continuous encouragement.

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Phenytoin Induced Toxic Epidermal Necrolysis (ten) in a Stroke

Patient: A Case Review

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Abstract

Background: Some of the serious cutaneous Adverse Drug Reactions (ADRs) include Stevens Johnson Syndrome (SJS), Toxic Epidermal Necrolysis (TEN), and overlap category of SJS and TEN. Drugs are considered as one of the most common causative factor for these serious ADRs especially with phenytoin. We present a patient who developed TEN after phenytoin treatment.

Case report: A 50yr old female was admitted in female general medicine ward in Gandhi Medical College & Hospital, Secunderabad with complaints of rashes all over the body and unable to swallow & food intake for past 2 days. She is a known case of CVA with IC bleeding and was prescribed with phenytoin for seizure one and half month before alongside regular medicine. She develops erythematous rash was all over the body (more than 70%) with mucosal (oral and conjunctival) involvement skin peeling of face. Aggressive symptomatic and supportive treatment was given for the management of TEN. Patient was discharged after 23days treatment in hospital

Conclusion: Although TEN is a rare toxicity it must always be considered during phenytoin therapy and such patient should be managed appropriately by implying standard guideline and by using procedure which have published previously so that patient care can be paramount.

Key words: ADRs, TEN, Phenytoin

Introduction:

Cutaneous drug eruptions are most occurring complication of adverse drug reactions (ADRs) [1]. It affects around 2-3% of all hospitalized patients. Drugs are considered as one of the most common causative factor for these serious ADRs [2]. Some of the serious cutaneous ADRs include Stevens Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and

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overlap category of SJS and TEN. TEN is a life-threatening serious muco-cutaneous illness associated with high fever and confluent erythema followed by necrolysis where two or more mucosal sites are usually affected; whereas SJS is characterized by presence of flat, atypical target lesions and epidermal detachment is < 10% of total body surface area (BSA). Flat, atypical target lesions may also be seen with TEN (TEN with spots) and sometimes, extensive necrolysis can occur without target lesions (TEN without spots). In SJS-TEN overlap category, epidermal detachment is 10-30% of the total BSA. These reactions are often associated with significant mortality [3]. The drugs commonly concerned as the cause of SJS/TEN are anticonvulsants, sulfonamides, non-steroidal anti-inflammatory drugs and antibiotics [4][5]. In this case report we will discuss phenytoin (anticonvulsant) induced TEN occurred at a tertiary care teaching hospital.

Case discussion:

A 50yr old female was admitted in the female general medicine ward in Gandhi Medical College & Hospital, Secunderabad on 28/12/2013 with complaints of rashes all over the body and unable to swallow & food intake for past 2 days.

On examination

Patient was-

- disoriented
- febrile
- erythematous rash was positive all over the body with mucosal (oral and conjunctival) involvement
- skin peeling of face was also found
- BP- 130/90 mmHg, pulse rate-98 beats/min, cardiovascular sound-

S1&S2 positive, lungs- bilateral air entry positive.

Past medical and medication history

A known case of hypertension for past 5 years and on antihypertensive medication but taking irregular medication & developed cerebro vascular accident (CVA) with intra cranial bleeding before one and half month (14/11/2013) along with weakness in both right upper & lower limb, deviation of mouth to left side, several episodes of vomiting and 2 episodes of seizures. She was prescribed with following medication for 30 days

1. Tab. Atorvastatin 20 mg od hs
2. Tab. Ramipril 5 mg bid pc
3. Tab. Amlodipine 5 mg od pc
4. Tab. Citicoline 500 mg bid pc
5. Tab. Phenytoin 100 mg 1tab in morning – 2tabs in night
6. Tab. Folic acid 5 mg od pc
7. Protein-X powder 2tsf bid mixed with milk

After review all information regarding the patient, she was provisionally diagnosed as: **?drug induced SJS may be due to Phenytoin.**

On admission she was prescribed with

1. Intravenous fluid; DNS 2 pint & RL 1 pint
2. Intravenous fluid; D25 tid
3. Inj. Dexamethasone 8mg iv bid
4. Lot. Calamine for local application
5. Tab. Atorvastatin 20 mg od hs
6. Inj. Ranitidine 50 mg iv bid
7. Inj. Optineuron (Vit. B-Complex) 3ml in 125 ml normal saline od
8. Inj. Ceftraxone 1gm iv bid
9. Inj. Paracetamol 500 mg iv bid

Laboratory test results

Shows abnormalities in Hemoglobin - 10.5 gm/dl (11.0 -16.5 gm/dl), RBC – $3.4 \times 10^6 / \text{mm}^3$ ($3.8 - 5.8 \times 10^6 / \text{mm}^3$), WBC – $11.0 \times 10^3 / \text{mm}^3$ ($3.5 - 10.0 \times 10^3 / \text{mm}^3$) and HCT – 31.4 % (35.0 – 44.1 %)

Same medication was continued for the next day. On day 3rd (30/12/2013) patient was referred to dermatologist for consultation and was finally diagnosed as **Phenytoin induced TEN** & advised to keep under medical care. Dermatologist added Soframycine cream (Framycetin Sulphate) bid & saline compress along with existing prescription. On day 4th (31/12/2013) patient was afebrile, disoriented and lip bleeding was positive. Paracetamol was removed and Zytee gel (Benzalkonium chloride + Coline salicylate) was added in the existing medications. From day 5th - day 11th (01/01/2014 to 07/01/2014) patient was stable, general condition was fair; bleeding was decreased and was responding to verbal communication. Same medications were continued for this period. On day 12th (08/01/2014) patient was normal and same medication was continued with addition of Tab. Vitamin C 500 mg od. Same treatment was continued for next day (13th day) also (09/01/2014). On day 14th (10/01/2014) patient general condition was fair and erythematous rash was cured so calamine lotion was stopped from the prescription. On the same day patient visited to the dermatologist again and was found to be bleeding present with lips other were normal at diagnosis and was prescribed freshly with following prescription.

1. Inj. Dexamethasone 4mg iv bid for 2days, followed by 4mg iv od for

2days, followed by 2mg iv od and stopped.

2. Cream Moizen (soft paraffin + light liquid paraffin), application over lips
3. Cream. Fusiderm-H (Fusidic acid + hydrocortisone), application over lips
4. Liquid paraffin, for eye application morning and evening.

From day 15th to 20th (11-01-2014 to 17-01-2014) patient general condition was fair with lip bleeding was continuous, and same prescription was continued by gradual decreasing the dexamethasone dose and by addition of cefotaxim 1gm iv bid, metronidazole infusion iv bid and ranitidine 150 mg iv bid. On day 21st (18-01-2014) patient re-visited dermatology department and was found to be recovering from the TEN with no fresh lesion present and hypo&hyper pigmentation all over the body. Patient was freshly prescribed with the following prescription.

1. Cream Moizen (soft paraffin + light liquid paraffin), application over lips
2. Cream. Fusiderm-H (Fusidic acid + hydrocortisone), application over lips
3. Liquid paraffin, for eye application morning and evening.
4. Tab. Ciprfoxacin 500 mg bid
5. Tab. Vitamin C 500 mg od

Same medication was continued for next day (22nd day) 19-01-2014 as patient was normal and ready for discharge. On day 23rd (20-01-2014) patient was fit to discharge as no further complains regarding skin eruption was present. And following discharge medication was prescribed for 2weeks and asked for revisit in dermatology out-patient.

1. Cream Moizen (soft paraffin + light liquid paraffin), application over lips

2. Cream. Fusiderm-H (Fusidic acid + hydrocortisone), application over lips
3. Liquid paraffin, for eye application morning and evening.
4. Tab. Pantoprazole 40mg od before breakfast.
5. Tab. Vitamin C 500 mg od
6. Tab. B-complex



Discussion:

As drugs are most common causes of SJS and TEN it is top most priority to identify the medication causing the condition and stopping the drug as early as possible. Necessary precautions should be adopted to prevent re-occurrence from unintended re-challenge [6]. Previous study also reported that, short term usage of phenytoin increase the risk of SJS and TEN for a period of less

than eight week. In such cases, offending drug should be withdrawn [7]. The time between first administration and development of SJS/TEN is 1-4 weeks in majority of cases [8]; this is same in this case, where TEN has developed due to phenytoin within 4 weeks of therapy.

Some report suggests steroids are treatment of choice in severe cases, to limit the inflammatory process, along with

prophylactic systemic and topical antibiotics. However, in cases of phenytoin reactions, carbamazepine, and phenobarbitone should be avoided as they can cross react in such patients [9]. Same as this case, where dexamethasone were used along with systemic antibiotics as the situation was critical and aggressive therapy were required to manage the situation.

SJS/TEN is a life threatening condition and therefore supportive care is an essential part of the therapeutic approach. A multicenter study conducted in the USA, and including 15 regional burn centers with 199 admitted patients, showed that survival rate - independent of the severity of disease (APACHE-score and TBSA=Total body surface area) - was significantly higher in patients who were transferred to a burn unit within 7 days after disease-onset compared with patients admitted after 7 days (29.8% vs 51.4% ($p < 0.05$)). This positive association of early referral and survival has been confirmed in other studies [10]. Same in our case patient got admitted within two days of onset of cutaneous adverse reactions which has saved the life of the patient.

Few other reports also suggests the treatment as mainly supportive with removal of the precipitating agent, good nursing care preferably on a ripple bed, care of the eyes and mouth to prevent scarring and infection and maintenance of fluid and electrolyte balance. Patient should be put on a high protein diet 2-3 gms/kg daily. Naso-gastric feeding is preferable in severely ill patients [8]. In this case patient was also provided with supportive therapy as cream for skin lesion and other bleeding. Fluid supplement and protein powder was also added in the

prescription as per reported, additionally appropriate nursing care as saline compresses for skin lesions and ryles tube was fitted for naso-gastric feeding.

Recovery is slow over a period of 14-28 days and relapses are frequent. There is a tendency for scarring in all but the mildest of cases. Mortality is 25%-50% and rises with age, being more than 50% above 60 years of age. Half the deaths occur due to secondary infection. Pulmonary edema, pulmonary embolism and gastrointestinal hemorrhage are other important causes of mortality. Reticulate skin pigmentation may occur over the affected areas [6]. In this case it reflects the same which is reported earlier, as it took total 23 days for the patient being discharge from hospital.

Conclusion:

Our case emphasizes that physicians should be aware of the potentially life threatening complications of phenytoin like TEN, which is so commonly used by them. And such patient should be managed appropriately by implying standard guideline and by using procedure which have published previously so that patient care can be paramount.

List of abbreviation:

BP – Blood Pressure.
Inj – Injection
Tab – Tablet
Lot – Lotion
mg – milligram
kg – kilogram
od – once in a day
bid – bis-in-die (two times a day)
pc – post cibos (after meal)
hs – horra somni (at bed time)
DNS – Dextrose Normal Saline
RL – Ringer's Lactate 25%

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Informed consent form:

Informed consent form obtained from the patient for publication. A copy of consent form is available with author for submission.

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Application of QSAR in Drug Design and Drug Discovery

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Abstract

Over past twenty years a large number of ligands, both agonists and antagonists have been developed by computational methodologies which are used to increase the efficiency of drug discovery process by rendering the design of new drug candidates. Both 2D and 3D-quantitative structure activity relation (QSAR) studies have been carried out using topological parameters along with thermodynamic and structural descriptors. The scope of this review is to highlight the use of pharmacophoric models and QSAR studies for identification and optimization of new ligands having potential to develop as drug candidates.

Key words: QSAR, pharmacophore, lipophilic parameters, electronic parameters, steric parameters

Introduction

The QSAR is based on structure activity relation (SAR) approach. It uses physicochemical properties (parameters) to represent drug properties that are believed to have a major influence on drug action. Some of the common pharmacophoric features include hydrophobic, aromatic, hydrogen bond acceptor, hydrogen bond donor, positive ionizable, and negative ionizable groups.

These parameters are properties that are capable of being represented by a numerical value which are used to produce a general equation correlating activity with relevant physicochemical properties. Many applications of QSAR and 3D QSAR methods have proved their utility in drug discovery. Once a 3D QSAR model is generated for a group of compounds having desired activity, it is further optimized by molecular docking to predict the interaction mode between the high/low potent ligands and protein ([enzyme/receptor](#))[1].

Parameters

1) Lipophilic parameters

Two parameters are commonly used to relate drug absorption and distribution with biological activity, namely, the partition coefficient (P) and the lipophilic substituent

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Constant (p). The former parameter refers to the whole molecule whilst the latter is related to substituent groups. A drug has to pass through a number of biological membranes in order to reach its site of action. Partition coefficients were the obvious parameter to use as a measure of the movement of the drug through these membranes. The nature of the relationship obtained depends on the range of P values for the compounds used. If this range is small the results may, by the use of regression analysis, be expressed as a straight line equation having the general form:

$$\text{Log}(1/C) = k_1 \log P + k_2 \dots\dots\dots (1)$$

where k_1 and k_2 are constants. This equation indicates a linear relationship between the activity of the drug and its partition coefficient.

2) Electronic parameters

The distribution of the electrons in a drug molecule will have an influence on the activity of a drug. In order to reach its target a drug normally has to pass through a number of biological membranes. As a general rule, non-polar and polar drugs in their unionized form are usually more readily transported through membranes than polar drugs and drugs in their ionised forms. Furthermore, once the drug reaches its target site the distribution of electrons in its structure will control the type of bonds it forms with that target, which in turn affects its biological activity. In other words, the electron distribution in a drug molecule will have an effect on how strongly that drug binds to its target site, which in turn affects its activity.

The distribution of electrons within a molecule depends on the nature of the electron withdrawing and donating groups

found in that structure. For example, benzoic acid is weakly ionised in water. Substitution of a ring hydrogen by an electron withdrawing substituent (X), such as a nitro group, will weaken the $O-H$ bond of the carboxyl group and stabilise the carboxylate anion. This will move the equilibrium to the right which means that the substituted compound is a stronger acid than benzoic acid ($KX > K$). It also means that at equilibrium more of the nitro benzoic acid will exist as anions, which could make its transfer through membranes more difficult than that of the weaker less ionised benzoic acid. Conversely, the introduction of an electron donor substituent (X) such as a methyl group into the ring strengthens the acidic $O-H$ group and reduces the stability of the carboxylate anion.

3) Steric parameters

In order for a drug to bind effectively to its target site the dimensions of the pharmacophore of the drug must be complementary to those of the target site. The Taft steric parameter (E_s) was the first attempt to show the relationship between a measurable parameter related to the shape and size (bulk) of a drug and the dimensions of the target site and a drug's activity. This has been followed by Charton's steric parameter, Verloop's steric parameters and the molar refractivity (MR), amongst others. The most used of these additional parameters is probably the molar refractivity. However, in all cases the required parameter is calculated for a set of related analogues and correlated with their activity using a suitable statistical method such as regression analysis. The results of individual investigations have shown varying degrees of success in relating the biological activity to the parameter. This is probably because little is known about the

finer details of the three-dimensional structures of the target sites.

Applications

1. Malaria, caused by the *plasmodium* parasite, is a major threat in the developing world, infecting 247 million people annually, and causing one million deaths. Resistance to anti-malarial drugs is a major public health problem to the control of malaria. Molecular docking is used to study how ligands are interacting with its biological target. Murray et al reported QSAR, pharmacophore and docking studies of dihydrofolate reductase thymidylate synthases inhibitors [2, 3]

2. Thrombotic disorders remain the major cause of death and disability in the western society and are projected to be the leading cause of death worldwide within last twenty years [4]. The use of anticoagulants in the treatment and prevention of both acute and chronic thrombosis-related disorders is growing at a rapid pace, in part due to an increasing geriatric population and the

recognition of intravascular diseases such as myocardial infarction, unstable angina, deep vein thrombosis, pulmonary embolism and ischemic stroke [5]. The significant role of thrombin makes it an attractive target in the design of new drugs for the treatment of cardiovascular and other diseases [6,7]. Fareed *et al.* [8] reported a development Status of Site Directed Thrombin Inhibitors (Table 1).

3. Cancer is a group of diseases characterized by the proliferation of cells without normal cellular controls over these events. These diseases are the second leading cause of death in the USA with approximately 1.2 million new cases diagnosed each year. With the revolutionary discoveries in molecular biology it became obvious that specific targets can be identified in tumors cells, the functions of which are necessary prerequisites for their replication. These targets might be specifically blocked by molecules designed and synthesized for this purpose. The

Table 1: Development Status of Site Directed Thrombin Inhibitors, Taken from Literature [8]

Agents	Chemical name	Developmental status
Hirudin, PEG-Hirudin and related variants	Recombinant analogues of natural Hirudin and their derivatives	Various clinical phases of development. Additional derivatives are being developed. One product is available in Europe.
Hirulogs	Synthetic bifunctional oligopeptides	Phase II and III clinical studies completed. Additional studies are carried out at this time.
Peptidomimetics	Synthetic heterocyclic derivatives	Phase II and III clinical development in the U.S.

advances in QSAR studies have widened the scope of rationalizing the drug design and, even finding the mechanisms of drug actions. QSARs have proved their worth in the interpretation of mechanisms of inhibition of a number of enzyme systems and a variety of anticancer drugs [9, 10].

4. Tyrosine kinase have emerged as new promising targets for cancer therapy [11]. Tyrosine kinase plays a central role in transformation of cells. This can be achieved in several ways; gene amplification and/or over expression of protein tyrosine kinase (PTKs) example, EGFR and erbB over expression that is commonly observed in several cancers, causes enhanced tyrosine kinase activity with quantitatively and qualitatively altered downstream signaling [12]. erbB-2 is involved in development and progression of several human cancers including lung and breast. The erbB family of receptors transmembrane receptor tyrosine kinase is involved in a wide range signal transduction and cellular functions, and has become a very fruitful area for the successful development of drugs to treat cancer [13]. erbB-2 is found to be significantly overexpressed in 20-30% of human breast cancer and is associated with poor prognosis [14]. The development of tyrosine kinase inhibitors has therefore become an active area of research in pharmaceutical science. One could not, however, confirm that the compounds designed would always possess good inhibitory activity to tyrosine kinase erbB-2, while experimental assessments of inhibitory activity of these compounds are time-consuming and expensive. Consequently, it is interesting to develop a prediction method for biological activities before the synthesis. QSAR searches information relating chemical structure to

biological and other activities by developing a QSAR model. Using such an approach one could predict the activities of newly designed compounds before a decision is being made whether these compounds should be really synthesized and tested.

5. The Epidermal Growth Factor (EGF) and its receptor have been identified as key drivers in the process of cell growth and replication. The kinase domain of EGFR is known [15] and provides a basis for structure based design; ligands based approach also provides an effective way for designing new inhibitors. There are few core structures which can be used for designing the best molecule. There are several EGFR, QSAR models published in the literature [16,17]. The models are based on Hansch analysis or 3D-QSAR techniques [14, 15].

6. Adenosine receptors (ARs) are a family of G-protein coupled receptors (GPCRs) of great interest as targets for therapeutic intervention. There is a lack of reliable adenosine receptor structures. So, adenosine ligands, agonists and antagonists, have been developed by homology modeling of GPCR. Homology modeling is a computational method for constructing a three-dimensional model of a protein from its amino acid sequence by means of the alignment with one or more known protein structures, namely templates, likely to resemble the structure of the query sequence [18, 19]. Once generated, a homology model could be used with structure-based techniques.

Discussion

Various compounds have occupied researchers in recent years and numerous computational models have been drawn up. Many of these models have been generated by means of Ligand-based approaches, mainly pharmacophore modeling and 3D

QSAR studies. Such models were capable to predict a potent drug for new drug discovery. Most of these models have also been successfully applied to the design of new ligands or to the optimization of known active compounds[20-24]. But the problems with the industrial application of QSAR stem from difficulties that arise in the following stages of modeling:

- ❖ data collection and accessibility
- ❖ determination of the error level of data
- ❖ presentation of molecular structure
- ❖ choice of the appropriate QSAR model
- ❖ optimization of model architecture
- ❖ identifying the optimal subset of variables
- ❖ robustness of model and the size of external validation effort.

There are many problems but there are also many recommendations which were given in 1973, by Unger and Hansch. They are

- ❖ Independent parameters should use.
- ❖ Parameters should be validated

Now a day, there are more recommendations for 3D QSAR. They are

- ❖ Select rationalized starting geometries.
- ❖ Cross validation should be done.
- ❖ Prediction of biological activity values depend on training set.
- ❖ Observed value and predicted value should be summarized.[25,26]

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A Review of Plants used as Contraceptives

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Abstract

This review presents information gathered on scientifically proved medicinal plants used for anti-fertility activity. This study provides the information on botanical name, family, parts used, and common name. In spite of rapid progress and spread of modern medicine and surgery, faith in and popularity of traditional methods has not decreased. There are a large number of studies which supports the anti-fertility effects of traditional herbal medicines. The aim of this review is to focused the work on anti-fertility of herbal medicines. This article may help investigators to identify medicinal plants responsible for anti-fertility activity.

Key words: anti-fertility plants, traditional herbal medicines, estrogenic activity and botanical name

Introduction

The population problem is one of the biggest problems facing the country, with its inevitable consequences on all aspects of development, especially employment, , housing, education, , health care, sanitation and environment. According to U.S. Census Bureau estimates, world population hit the six billion in June 1999. This figure is over 3.5 times the size of human population at the beginning of the 20th century.

The time required for global population to grow from 5 to 6 billion, which took 12 years was shorter than the interval between any of the previous billion. In 2005 G.C., world population is estimated to be 6.5 billion. This number is expected to increase by 2.5 billion over the next 45 years, 6.5 billion to 9.1 billion in 2050. Today, 95 per cent of all population growth is absorbed by the developing world and 5 per cent by the developed world.[1]

The World Health Organization (WHO) has set up a Task Force on Plant Research for fertility regulation with an objective to find new orally active non-steroidal contraceptive compounds. Various medicinal plant extracts have been tested for their anti-fertility activity both in male and

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female. Some of these plants had spermicidal and altered hormone levels. It is necessary to use biologically active botanical substances or fertility-regulating agents of plant origin which are eco-friendly. The average birth per woman is 6.14 and the contraceptive prevalence is 8.1 percent[2,3]. Contraception is literally the prevention of conception, but generally is taken to mean the prevention of pregnancy. Family planning has been promoted through several methods of contraception, like contraceptive pills, Copper-T, Diaphragm Tubectomy, Condoms, and coitus interrupts. These methods are mostly female oriented. Contraceptive pills contain usually female sex hormone like estrogen, progesterone or their derivatives single or together. Novid was the first “pill” approved by FDA for use as contraceptive agent in the USA in 1959. But unfortunately these pills develop some unwanted effects like Hypertension, obesity, dysmenorrhea, vomiting, cardiovascular disorders and carcinoma of breast and uterus. So these pills are not safe for long term use.

Various measures have been taken to minimize the side effects of these pills but there is little success. Due to serious adverse effects produced by synthetic steroidal contraceptives, attention has now been focused on indigenous plants for possible

contraceptive effect. Although contraceptives containing estrogen and progesterone are effective and popular, the risks associated to the drugs have triggered the need to develop newer molecules from medicinal plants.

From the advancement of reproductive biomedicine, several hormonal contraceptive pills have been developed but no one is free from different side effects. For this purpose, the World Health Organization (WHO) has constituted a population control programme, which includes studies having traditional medical practices. At present global attempt has been taken to search out the effect of herbal product for contraceptive purposes[4,5].

The review of literature in this regard has included plants having folkloric reputations and those plant extracts which have shown to be active in animals as well as in humans as antifertility drugs at various stages of pregnancy like estrogenic agents, uterine stimulants, abortifacients, antiimplantation effects, Abortifacient activity, Contraception activity etc. Search or survey of medicinal folklore that to in relation to birth control or contraception in particular is a herculean task.[6]

Some medicinal plants having antifertility activity below the tables

Medicinal Plants having Antiimplantation activity

SL No.	Common Name	Botanical Name	Family	Parts Used	References
1.	Barna and Varuna	Crataeva nurvala	Capparidaceae	Stem and Bark	18
2.	Somjava / Jewels of opar	Talinum Paniculatum	Dioscoreaceae	Root and Leaf	14
3.	Lasura	Cordia dichotoma	Boraginaceae	Bark	26

4.	Desert Horse Purslane	<i>Trianthema portulacastrum</i>	Aizoaceae	Stem Leaf	27
5.	Wood Sorrel	<i>Oxalis corniculata</i>	Oxalidaceae	Whole Plants	14
6.	Nag Champa	<i>Artabotrys odoratissimus</i>	Annonaceae	Leaf	28
7.	Kaila Spati	<i>Couroupta guianensis</i>	Lecythidaceae	Bark and Flower	28
8.	Moss Rose / Pursley	<i>Portulaca oleracea</i>	Portulacaceae	Aerial Parts	29
9.	Jiwanti / Dodi	<i>Leptadenia reticulata</i>	Asclepiadaceae	Whole Plants	30
10.	Haldi	<i>Curcuma aromatica</i>	Zingiberaceae	Rhizome	14
11.	Banda	<i>Dendrophthoe falcata</i>	Loranthaceae	Aerial Parts	4
12.	Nata Karanja	<i>Caesalpinia bonduie</i>	Caesalpinaceae	Root and Bark	31
13.	Golden Shower	<i>Cassia fistula</i>	Caesalpinaceae	Seeds	18
14.	<i>Calliandra brevipes</i>	<i>Derris brevipes</i>	Papilionaceae	Root	4
15.	Water Willow	<i>Justicia simplex</i>	Acanthaceae	Root	21

Medicinal plants having Abortifacient activity

SL No.	Common Name	Botanical Name	Family	Parts Used	References
1.	Indian Tree of Heaven	<i>Ailanthus excels</i>	Simaroubaceae	Stem Bark	18
2.	Hopbush	<i>Dodonea Viscosa</i>	Sapindaceae	Aerial Parts	32
3.	Aaghada	<i>Achyranthes aspera</i>	Amaranthaceae	Root	14
4.	Betel Nut	<i>Areca catechu</i>	Arecaceae	Nut	33
5.	Danti	<i>Jatropha curcas</i>	Euphorbiaceae	Fruit	4
6.	Pursley	<i>Portulaca oleracea</i>	Portulacaceae	Aerial Parts	29
7.	Desert Hors Purslane	<i>Trianthema portulacastrum</i>	Aizoaceae	Stem, Leaves, Roots	27
8.	Common Rue	<i>Ruta graveolens</i>	Rutaceae	Aerial Parts	14
9.	<i>Calliandra brevipes</i>	<i>Derris brevipes</i>	Papilionaceae	Root	4

Medicinal Plants having Contraception activity

SL No.	Common Name	Botanical Name	Family	Parts Used	References
1.	Desert Date	Balanites roxburghii	Zygophyllaceae	Fruits	14
2.	Neem	Azadirachta indica	Meliaceae	Seed	14
3.	Common Night Glory	Rivea hypocrateriformis	Convolvulaceae	Aerial Parts	18
4.	Malai Vembu	Melia azedarach	Meliaceae	Aerial Parts	18
5.	Danti	Jatropha curcas	Euphorbiaceae	Fruits	4
6.	Common Rue	Ruta graveolens	Rutaceae	Aerial Parts	14
7.	Long Piper	Piper longum	Piperaceae	Seed	18,27
8.	Pudina	Mentha arevensis	Lamiaceae	Leaf	5
9.	Bilva	Aegle Marmelos	Rutaceae	Leaf	18,12
10.	Brahmi	Bacopa monnieri	Scrophulariaceae	Plant	18

Medicinal Plants having Antiovalatory Activity

SL No.	Common Name	Botanical Name	Family	Parts Used	References
1.	Betel Nut	Areca catechu	Arecaceae	Nut	33
2.	Dhak ki-be	Rivea hypocrateriformis	Convolvulaceae	Aerial Parts	18

Medicinal Plants having Estrogenic Activity

SL No.	Common Name	Botanical Name	Family	Parts Used	References
1.	Desert Date	Balanites roxburghii	Zygophyllaceae	Fruit	14
2.	Jiwanti / Dodi	Leptadenia reticulata	Aselepiadaceae	Whole Plants	30
3.	Fenzl	Momordica cymbalaria	Cucurbitaceae	Root	14
4.	Ambushi	Oxalis corniculata	Oxalidaceae	Whole Plant	14
5.	Calliandra	Derris brevipes	Papillioraceae	Root	4

	brevipes				
6.	Hausa	Spondias mombin	Anacardiaceae	Leaf	8
7.	Chinarose	Hibiscus rosa sinensis	Malvaceae	Root	4
8.	Pala indigo	Wrightia tinetoria	Apocynaceae	Stem Bark	7
9.	Sodom Apple	Calotropis procerra	Asclepradaceae	Root	34

Medicinal Plants having Anti-Estrogenic Activity

SL No.	Common Name	Botanical Name	Family	Parts Used	References
1.	Callindra-brevipes	Derris brevipes	Papillioraceae	Root	4
2.	Aaghada	Achyranthes aspera	Amarantraceae	Root	14
3.	Betel Pepper	Piper betel	Piperaceae	Petiol	14
4.	Fenugreek	Trigonellafoenum gracum	Fabaceae	Seed	14
5.	Lotus	Nelumbo Nucifera	Nymphacacea	Seed	20
6.	Honeysuckle Mistletoe	Dendrophthoe falcate	Loranthaceae	Aerial Parts	4
7.	Ambusi	Oxalis corniculata	Oxalidaceae	Whole Plant	14
8.	Nata Karanja	Caesalpinia bonduc	Caesalpinaceae	Root and Bark	31

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A Novel Class of Gene Delivery Systems: Exosomes

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Abstract

Biological therapeutics, including short interfering RNA and recombinant proteins, are prone to degradation, have limited ability to cross biological membranes, and may elicit immune responses. Therefore, delivery systems for such drugs are under intensive investigation. Exploiting extracellular vesicles as carriers for biological therapeutics is a promising strategy to overcome these issues and to achieve efficient delivery to the cytosol of target cells. Exosomes are biological membrane vesicles measuring 30 to 100 nm. They contain an abundance of small molecules like tetraspanins, receptors for targeting and adhesion, lipids, and RNA. They are secreted by most biological cells, and are involved in a plethora of physiological functions including, but not limited to, transport of genetic material, modulation of the immune system, and cell-to-cell communication. Due to their viral-like transfection efficiency and inherent biological function, compelling evidence indicates that exosomes can be used as novel delivery platforms for gene therapy. This review provides insights into the composition and functional properties of exosomes, and focuses on therapeutic, diagnostic and gene delivery potential of exosomes.

Key words: Exosomes, RNA, Gene therapy, extracellular vesicles.

Introduction

Cells are well known to communicate via soluble mediators or cell-cell contact, but in recent decades, intercellular communication through extracellular vesicles has also increasingly gained attention.

The first notion of such vesicles arose when Wolf described the formation of “platelet dust” upon storage of blood platelets [1].

These phospholipid-rich particles were shown to exert coagulant activity and were later determined to be actively shed membrane-derived vesicles [2]. Since then our knowledge about such vesicles has expanded dramatically, and vesicle secretion is now widely accepted to occur in most, if not all, cell types. Characterization studies identified three main populations of extracellular vesicles, which are commonly classified based on their intracellular origin.

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Cells that undergo apoptosis fractionate their cellular content into subcellular apoptotic bodies in order to prevent leakage of possibly toxic or immunogenic cellular contents into the extracellular matrix (Figure 1) [3]. Apoptotic bodies appear as a heterogeneous group of vesicles, with sizes ranging from 50 nm to 5 µm and a buoyant density of 1.16–1.28 g/mL [4–7]. They contain a variety of cellular contents, including DNA, RNA, and histones, and display “eat-me” signaling molecules, causing them to be rapidly cleared by macrophages [8, 9]. Due to their specific

A microvesicles are supposedly generated by budding from the plasma membrane, exosomes appear to be formed by tightly controlled inward budding into large

cellular content and high density, they may be distinguished from two other major vesicle populations, which show considerably more overlap. One of these populations originates from budding and fission from the plasma membrane into the extracellular space (Figure 1, middle panel) and contains vesicles of about 50–1000 nm in size. Such vesicles are interchangeably referred to as microvesicles [10], ectosomes [11], shedding vesicles [12], icroparticles [13,14], plasma membrane-derived vesicles [15] or even exovesicles[16].

(Figure 1) In theory, exosomes and microvesicles are clearly distinguishable by their origin, but in practice such a distinction is seldom possible [17].

Exosomes are bio-nanoparticles, measuring

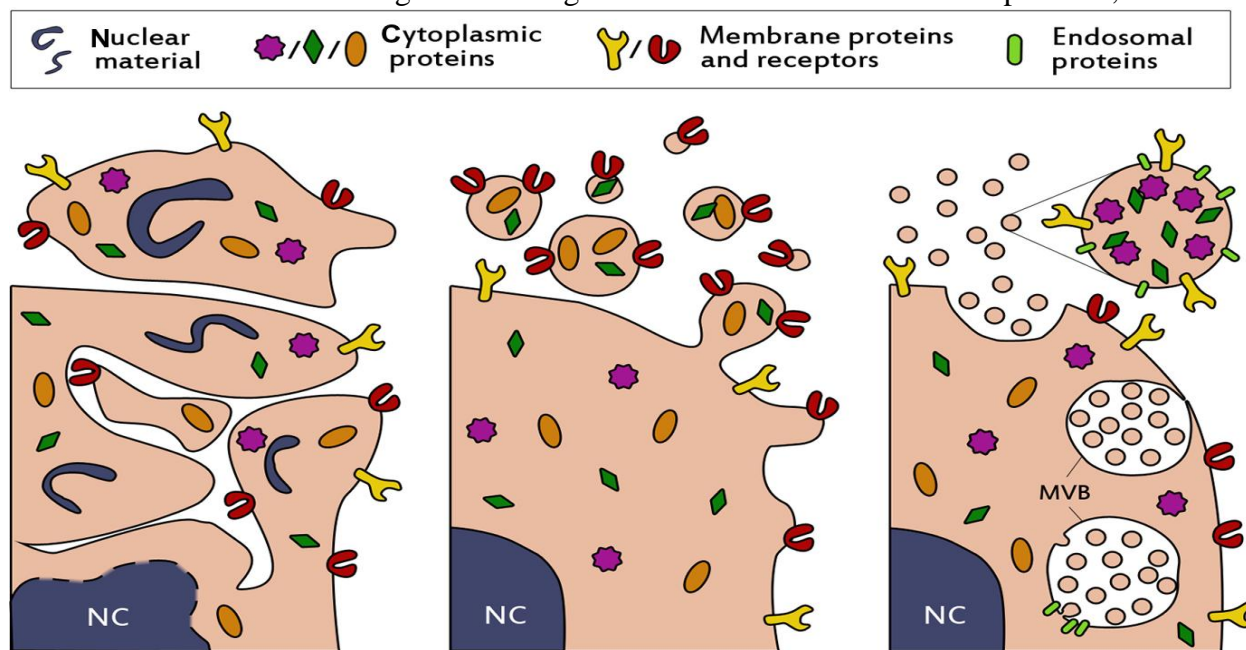


Figure 1: Schematic presentation of the biogenesis and composition of the three main classes of extracellular vesicles.

multivesicular bodies in the cytosol. These multivesicular bodies are able to fuse with the plasma membrane, causing the release of exosomes into the extracellular space

30 to 100 nm in diameter, and are secreted by most biological cells [18]. They are responsible for a plethora of biological functions, including but not limited to, cell-

to-cell communication, signal transduction, transport of genetic materials, and modulation of immune responses [19]. Alluding to their nanometer size and inherent biological role [20], compelling evidence indicates that exosomes can be utilized as a novel nanoscale delivery platform for gene therapy [21], as well as a diagnostic tool for biomarkers of disease [22]. This dual functionality of therapeutics and diagnostics is termed “theranostics”, and is a new and emerging field of nanomedicine [23]. Gene therapy is a technique by which an abnormal gene is replaced with a normal one to correct the disease manifestation, and to restore biological function. However, the field of gene therapy is still in its infancy, and it has been fraught with setbacks in the past [24]. At the time of writing, the US Food and Drug Administration (FDA) has not yet approved any human gene therapy product for sale [25]. Interestingly, China has sought to lead the way by approving the world's first gene therapy-based drug, Gendicine™ (a recombinant adenovirus) in 2004 for use in cancer patients [26], with moderate success [27].

Exosomes

Exosomes are nanoscale membrane vesicles first described in the 1980s [28], but the word “exosome” was (confusingly and rather unfortunately) re-used in 1997 to refer to exoribonuclease complexes in RNA processing [29]. Exosomes are secreted by cells from multivesicular endosomes (Fig. 2), and transport various biological molecules, ranging from membrane receptors, proteins, to mRNA and (microRNA) miRNA for maintenance of biological homeostasis [30], as well as epigenetic reprogramming [31]. Exosomes

are constitutively generated (a process which is not calcium triggered) from late endosomes, which subsequently form multivesicular bodies, and it has been found that this mechanism is ceramide-dependent [32]. Upon release, exosomes can transport materials to neighboring cells via clathrin-mediated endocytosis [33]. However, there is also evidence to suggest that the uptake of exosome relies on specific surface molecules on the exosome itself, as well as the presence of specific receptors on the recipient cell membrane [34]. Due to the similarity in size and composition of retroviruses and exosomes, it has been postulated that exosomes can serve as the ultimate “Trojan horse” for delivering small molecules into cells (similar to how viruses infect cells) [35], with a potential for being gene delivery vehicles.

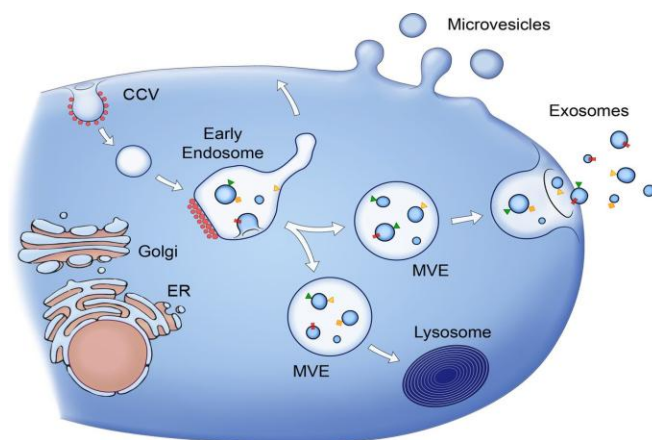


Figure 2: Release of MVs and exosomes.
Functions of exosomes

Physiological

Exosomes are involved in the modulation of the immune system by functioning as shuttles for antigen presentation [36]. Upon internalization of exosomes, antigen-specific

immune responses can then be appropriately mounted [37]. Dendritic cells can also secrete exosomes to communicate with T cells and B cells to mount an immune response or to mediate immune tolerance [38]. Thus, exosomes can either have a stimulatory or inhibitory effect on the immune system, although its exact mechanism has not been fully elucidated. Apart from its role in the immune system, it has also been demonstrated that exosomes serve other physiological purposes as well. Evidence suggests that mesenchymal stem cells secrete exosomes, which can attenuate ischemia reperfusion injury in myocardial infarction [39]. In addition, exosomes are also thought to be involved in the regulation of neuronal cell function [40,41] as well as communication between cells [42].

Pathological

Recent evidence suggests a stark similarity in both composition, as well as mechanism of action of material transfer in exosomes and retroviruses [43, 44]. Both exosomes and retroviruses have a lipid bilayer membrane, share a common glycan coat, and are enriched with similar proteins and genetic materials. It has been hypothesized that exosomes and retroviruses share a common ancestry, merely differing by a mutation of a single structural gag gene. Indeed, it has been shown that exosomes are involved in the functional delivery of viral miRNA, as a potent mechanism of infectivity [45]. It has been experimentally demonstrated that exosomes are associated with the transmission of prion proteins via intercellular membrane exchange in Creutzfeldt-Jakob disease [46–48].

Gene therapy

The concept of gene therapy is attractive to both the scientific community as well as

patients, because the idea of replacing a defective gene with a corrected one appears simple and elegant. However, the gene therapy fraternity was forced to re-evaluate its protocols with the sudden death of Jesse Gelsinger, the first patient in a gene therapy clinical trial to succumb to an acute immunological reaction to the adenovirus, which was used as a vector for delivering the gene [49]. Nevertheless, much progress has been achieved in the realm of gene therapy. Clinical trials have shown that gene therapy is safe and effective in treating diseases in humans such as severe combined immunodeficiency (SCID) [50], β -thalassemia [51], inflammatory bowel disease [52], Duchenne muscular dystrophy [53], and chronic lymphoid leukemia [54].

Conclusion

The safe and effective delivery of drug molecules to their target site is a field which has increasingly gained attention in drug design and development. In recent decades, the focus has shifted from synthetic drug compounds to the delivery of biological drugs (ie, proteins and nucleic acids), which are very prone to immune effects and degradation. In this regard, exosome mimetics are promising candidate delivery vehicles, given that they mimic nature's delivery vehicles of biologicals, but are not as complex as their biological counterparts. These characteristics may allow them to deliver biological in an effective and safe manner, with high pharmaceutical acceptability due to their well characterized components. This review provides insights into the composition and functional properties of exosomes, and focuses on therapeutic, diagnostic and gene delivery potential of exosomes. The similar mechanism of actions for gene transfection

of both exosomes and viruses highlights the potency of exosomes in gene therapy. As exosomes can be derived from the patient's own cells, the issue of immunogenicity can

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be circumvented. Nevertheless, the concept of utilizing exosomes as a gene delivery vehicle is an attractive and promising technique in gene therapy.

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Some Indian Vegetables used as Anticancer Agent

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Abstract

Cancer is the second leading cause of death worldwide. Conventional cancer therapies cause serious side effects and at best, merely extend the patient's lifespan by a few years. Cancer control may therefore benefit from the potential that resides in alternative therapies. The demand to utilize alternative concepts or approaches to the treatment of cancer is therefore escalating. However, the potential treatment of cancer is still under investigation. In fact the plants occupy a good place in the treatment. With the advanced knowledge of molecular science and the refinement in isolation and structural elucidation techniques, we are now in much better position to identify various anticancer plants. The medicinal plants and their products, particularly vegetables have antioxidant activity leading to anti cancer effect. Many doctors recommend that people wish to reduce the risk of cancer must eat vegetables everyday in their diet. The vegetables contain many phytochemicals having antioxidant activity. The antioxidant protects the cells from damaged caused by free oxygen radicals. Here the present article gives a better therapeutic approach to cancer by the maximum use vegetables against different cancers. **Key words:** Cancer, alternate therapies, antioxidant, phytochemicals

INTRODUCTION

The natural approach to treating cancer should be first, not the last method of treatment. Cancer patients are always confronted by seemingly impossible dilemmas where the available choices seem less than optimal.

The tendency is to put one's faith in an outer authority, such as an oncologist or even natural healer. However, the first order of business is to realize that these people are only allies, and that ultimately we must take responsibility and charge of our own decision. To do this we require a certain amount of soul searching solitude. If at all possible, providing there is a window of time and opportunity, embark on a natural treatment approach before committing to heroic measures such as surgery, chemotherapy and radiation. The reason is

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that these latter injure the body's immune healing capabilities and may impair any opportunity for natural healing to occur. Further, one needs to weigh carefully the question of life extension versus life quality [1].

The plant kingdom serves as a food and medicinal source, and thus maintains the vitality of human beings as well as animals without causing any toxicity. India is the largest producer of medicinal plant and is rightly called as "Botanical garden of the world". In the indigenous or traditional system of medicine, many medicinal plants and their preparation are now used for the treatment of different diseases, including cancer [2]. More than 50% of all modern drugs in clinical use are of natural products, many of which have been recognized to have the ability to include apoptosis in various tumour cells [3]. Medicinal plants, including vegetables are known to have good immunomodulatory antioxidant activities, leading to anticancer effect. They act by stimulating both non specific and specific immunity and may promote the host resistance against infection by re-stabilizing body equilibrium amid conditioning the body tissue. Hence the consumption of vegetables is widely accepted as lowering the risk of different type of cancer. Vegetables contain several phytochemicals having potent antioxidant activities. The antioxidant vegetables prevent from cancer by protecting cells from damaged caused by free radicals. Thus the consumption of diet rich in vegetables with antioxidant activity may protect from the occurrence of cancer [4].

This review article contains 26 anticancer vegetables which have been described ahead. The general data of these plants have been collected from some books, website,

and journals. However, the particular phytoconstituent (phytochemicals) present in these plants, their mechanism of action and uses against the various cancers as reported by different authors have been cited under each medicinal plant/herb. Many reports describe that the anticancer activity of the medicinal plants is because of the presence of certain phytoconstituents, which possess strong antioxidant activities. The main phytoconstituent antioxidants with anticancer activity includes vitamins (e.g., A, C, E and K), carotenoids or carotene, terpenoids, flavonoids, polyphenols, enzymes, minerals, polysaccharides, alkaloids, saponins, lignins, xanthonenes and certain pigments [5].

SOME IMPORTANT ANTICANCER VEGETABLES USED IN DIET

Beet root

(Biological source: - *Beta vulgaris* Family:

- Amaranthaceae)

The beet root contains FDA approved red food color E162, which can be effective in suppressing the development of multi-organ tumors in experimental animals. It decreases the growth rate of the PC-3 cells (androgen-independent human prostate cancer cells). Beetroot extract have showed significant cytotoxic effect normal human skin FC and liver HC cell lines. Betanin, the major betacyanin constituent, may play an important role in the cytotoxicity exhibited by the red beetroot extract [6]. The molecular components of a phenolic fraction of beta vulgaris were found to be vitexin-2''-O-rhamnoside, its demethylated form 2''-xylosylvitexin, isorhamnetin 3-gentiobioside, and rutin. The phenolic fraction inhibited MCF-7 cell proliferation.

Vitexin-2''*O*-rhamnoside strongly inhibited DNA synthesis in MCF-7 cells, whereas 2''-xylosylvitexin and isorhamnetin 3-gentiobioside were activators; combinations of activators and inhibitors maintained the over-all inhibitory effect [7]. In beet root vitamin C content was found to be 33,840 mg/100g. and in juices it was found to be 68 mg/100ml suc. The reaction speed of DPPH of beet root juice was found to be 5087 μ M/s [8].

Bitter gourd

(Biological source: - *Momordica charantia*)

Family: - Cucurbitaceae)

Administration of bitter gourd significantly reduced the incidence of ACF (Aberrant Crypt Foci). The ability of bitter gourd to reduce the incidence of ACF may be due to the compound momordin, which is found in bitter gourd. It was also observed that rats fed with bitter gourd had higher activities of hepatic detoxification enzymes (GST) and antioxidant enzymes (SOD and CAT). The treatment groups had significantly higher GST, SOD and CAT activity compared to the control. The GSH levels in the rats fed with bitter gourd were also found to be significantly higher compared to the control. GSH is utilized by GST as a substrate in the detoxification process. Superoxide dismutase catalyzes the dismutation of superoxides, which are potent carcinogens. The animal groups fed 2 and 4% bitter gourd diets had significantly higher CAT and SOD activities. It was observed that bitter gourd had phenolic content, indicating that it may play a role in the cancer prevention as the results from the animal study showed. Reduction of ACF by bitter gourd in the animal study could have been due to its antioxidative potential [9]. Ribosome-

inactivating proteins (RIP) of bitter gourd displayed strong apoptosis-inducing activity and suppressed cancer cell growth [10].

Brinjal

(Biological source: - *Solanum melongena*)

Family: - Solanaceae)

Brinjal peels extracts contains anthocyanins, delphinidin 3RGcaf5G showed the highest radical-scavenging activities toward both 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical and linoleic acid radical [11]. The topical application of cream Curaderm containing SRGs (solasodine rhamnosyl glycosides) obtained from egg plant is amazingly effective for treating large nonmelanoma skin cancer with incredible cosmetic results [12].

Broccoli

(Biological source: - *Brassica oleracea* var.

botrytis **Family: - Brassicaceae)**

Broccoli contains sulforaphane (SFN) induces time- and dose-dependent decline in survival of barrett esophageal adenocarcinoma cells (BEAC). SFN increases intracellular accumulation of drug in BEAC cells. SFN significantly enhances the antiproliferative effect of chemotherapeutic and telomerase-inhibiting agents in BEAC cells. SFN inhibits cell cycle progression and enhances the ability of paclitaxel to induce cell cycle arrest [13]. The anticancer activity of broccoli was the highest, and the IC₅₀ value of the extract inhibiting on the growth of A549, LAC, HELA and HepG2 were 14.38 ± 0.35 , 10.38 ± 0.34 , 19.45 ± 1.72 and 26.75 ± 0.82 mg/g, respectively. 3-BITC (3-butenyl isothiocyanate) and sulforaphane were found

as the major isothiocyanates in broccoli for anticancer activities [14]. Selenium-enriched broccoli sprouts could potentially be used as an alternative selenium source for prostate cancer prevention and therapy [15].

Bottle gourd

(Biological source: - *Lagenaria siceraria*

Family: - Cucurbitaceae)

The methanolic extracts of *Lagenaria siceraria* significantly inhibited the tumor volume, packed cell volume, tumor (viable) cell count. There is delayed in cell division and thereby increased in survival time in the animal studies. The oral administration of *L. siceraria* restore the haemoglobin content and maintain the normal values of RBC and WBC and supports its haematopoietic protecting activity. The level of lipid peroxide was reduced to normal which was elevated during cancerous stage, when treated with extract of *L. siceraria*. This reflects the decrease in free radical production and the subsequent reduction in oxidative stress. Reduced glutathione was significantly improved to normal which was reduced during disease stage [16].

Cabbage

(Biological source: - *Brassica oleracea*

Linne (*capitata* var. *alba* L.) **Family:** -

Cruciferae).

The cabbage (*Brassica oleracea*) was cultivated with supplementation of sulphur salts; as a result there is increase in total glucosinolates contents. The antioxidant properties of these sulphur supplemented cabbage was also higher than that of the normal sprout due to the increases of

phenolic compounds. Consequently, the glucosinolates fortified sprout has higher anti-proliferative activity against HepG2 human hepatocarcinoma cells than the normal sprout and the cell viability decreased by 22–35%. Also in CT26 mouse colorectal cancer cells, the cell viability decrease by 34–59% [17].

Carrots

(Biological source: - *Daucus carota* Family:

- Apiaceae)

The treatment of leukemia cell lines with carrot juice extract induces apoptosis and inhibits the progression through the cell cycle. Lymphoid cell lines were affected to a greater extent than myeloid cell lines and normal hematopoietic stem cells were less sensitive than most cell lines [18]. Carrot having phenylpropanoids extract 2-epilaserine shows significant cytotoxicity against HL-60 [19]. The potential anti-cancer effect of falcarinol extract of carrot may be due to the enhancement of the immune system which stimulates the production of T-lymphocytes and inactivates proteins/enzymes that are responsible for the proliferation of cancer cells [20].

Cauliflower

(Biological source: - *Brassica oleracea*

Family: - Brassicaceae)

3, 3'-Diindolylmethane (DIM), an indole derivative produced on consumption of *Brassica oleracea* can inhibit vascular endothelial growth factor (VEGF)-induced cell proliferation and DNA synthesis in human umbilical vascular endothelial cells (HUVECs). Consistent with this inhibition, VEGF-induced extracellular signal-

regulated kinase (ERK1/2) phosphorylation was greatly reduced. DIM inhibits RAS signaling induced by VEGF and other growth factors, which interferes with its downstream biological effects necessary for angiogenesis [21].

Chives

(Biological source: - *Allium schoenoprasum*

Family: - Amaryllidaceae)

Chives do exhibit anticancer, anticlotting, hypolipidemic, antibacterial, antiviral, and decongestant properties, but they are somewhat weaker than the properties of onions. The following volatile components have been identified: dipropyl disulfide, methyl pentyl disulfide, pentanethiol, penthyl-hydrodisulfide and cis/trans-3, 5-diethyl-1, 2, 4-trithiolane. Chives also contain significant amounts of the vitamin A and C. Chives are rich in a number of vitamins and minerals, which strengthen the immune system and improve the overall health. Antioxidants present in chives reduce the harmful effects of free radicals on the body, and protects from various diseases, even cancer [22].

Drumstick

(Biological source: - *Moringa oleifera*

Family: - Moringaceae)

Methanolic extracts of Moringa is having greater anticancer activity with ID₅₀ value of 0.32 µg mL⁻¹. Neutral red dye uptake assay showed drastic antiproliferation of cells with less dye uptake that refers to the active participation of methanol extracted compounds present in Moringa leaves [23]. *M. oleifera* ethanolic extract has the highest antioxidant activity at 77% inhibition of

radical formation. This high antioxidant capacity may be due to the high concentration of phenolics and flavonoids in *M. oleifera* extracts. A remarkable destruction of lymphoblast was found in treatment of methanolic extract on acute myeloid leukemia cells and acute lymphoblastic leukemia cells [24]. The methanolic extract of Moringa shows the strong scavenging effect in DPPH radical and reducing power assay. The methanolic extract of Moringa shows stronger hydrogen peroxide scavenging activity and higher SOD activity [25]. The aqueous extract of *M. oleifera* activates the apoptotic pathway in HeLa cells. The key bioactive compounds present in the aqueous fraction show a good anticancer activity and are relatively non-toxic to the normal healthy lymphocytes [26].

Garlic

(Biological source: - *Allium sativum*

Family: - Amaryllidaceae)

Garlic has anticancer activity against WEHI-164 tumor cells but the activity reduces on heating. The anticancer activities of different kinds of garlic are related to the level of allicin, flavanoids, and phenolic components. Therefore, fresh garlic has the highest content of bioactive components and the greatest anticancer efficacy²⁷. Diallyl disulfide (DADS), a sulfur compound derived from garlic shows anti-proliferative effects on colon cancer HT-29 cells [28]. Diallyl- and dipropyl- tetra sulfides have emerged as interesting irreversible inhibitors of the CDC25 (cell division cycle 25 phosphatases) isoforms A and C in-vitro. Furthermore, growth of both sensitive (MCF-7) and resistant (VCR-R) human

breast carcinoma cells was significantly decreased by these tetra sulfides. The observed antiproliferative effect arrests a G2-M cell cycle [29].

Ginger

(Biological source: - *Zingiber officinale*

Family: - Zingiberaceae)

Antioxidant activities found in ginger increases with increasing CO₂ concentration. Enriched ginger extract (rhizomes) exhibits the highest anticancer activity on MCF-7 cancer cells [30]. Ginger is an excellent source of several bioactive phenolics, including non-volatile pungent compounds such as gingerols, paradols, shogaols and gingerones. Ginger has been known to display anti-inflammatory, antioxidant and antiproliferative activities, indicating its promising role as a chemopreventive agent. Whole ginger extract (GE) exerts significant growth-inhibitory and death-inductory effects in a spectrum of prostate cancer cells [31]. 6-Shogaols, active constituent of ginger inhibits phorbol 12-myristate 13-acetate (PMA) - stimulated MDA-MB-231 breast cancer cell invasion with an accompanying decrease in matrix metalloproteinase-9 (MMP-9) secretion. 6-Shogaol was identified to display the greatest anti-invasive effect in association with a dose-dependent reduction in MMP-9 gene activation, protein expression and secretion. The NF-κB transcriptional activity was decreased by 6-shogaol. In addition, 6-shogaol was found to inhibit JNK activation [32].

Lady finger

(Biological source: - *Abelmoschus esculentus* (L.) Moench. **Family:** - Malvaceae)

The ripe fruits of lady finger contain quercetin, hyperin (hyperoside), hydrolysate of precipitated mucilage, proanthocyanidins, D-glucose, D-glucuronic and galacturonic acids which helps in anticancer activity. Fatty fraction of the fresh watery extract of the seeds causes destruction of cancerous cell growth *in vitro*. The pods are reported to exhibit antitumour activity [33]. The inhibitory effect of polysaccharides extracted from okra, *Abelmoschus esculentus* (L.) Moench was investigated on different human cancer cell lines, OVCAR-3, MCF-7, Hela and MCG-803 cells. Raw polysaccharide (RPS) had a significant inhibition effect on the proliferation of OVCAR-3 cells in a dose-dependent manner, and the lowest survival rates were 72.30% and 52.31%, respectively [34].

Little gourd

(Biological source: - *Coccinia grandis*.

Family: - Cucurbitaceae)

The antioxidant principles present in *Coccinia grandis* causes the reduction of Fe³⁺/ Ferricyanide complex to the ferrous form, and proves to be good antioxidant activity. *C. grandis* scavenges hydrogen peroxide in presence of phenolic groups and thereby neutralizes into water. Nitric oxide (NO) is a free radical which plays an important role in the pathogenesis of pain, inflammation, etc. *C. grandis* decreases the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro*. This may be due to the antioxidant principles which compete with oxygen to react with NO and inhibits the generation of nitrite [35].

Neem

(Biological source: - *Azadirachta indica*

Family: - Meliaceae)

Aqueous *Azadirachta indica* leaf extract significantly reduce the tumor incidence (33%), tumor multiplicity (42%), and increase in survival (34%) upon administration of Aqueous *A. indica* leaf extract to N-nitrosodiethylamine(NDEA)-abused mice [36]. Ethanol extract of neem leaves contains 2',3'-dehydrosalannol, 6-desacetyl nimbinene, and nimolinone. Treatment of C4-2B and PC-3M-luc2 prostate cancer cells with ethanolic extract inhibits the cell proliferation. The suppression of tumor growth is associated with the formation of hyalinized fibrous tumor tissue and the induction of cell death by apoptosis [37]. Azadirachtin, active constituent of neem, interacts with retinoic acid receptors and suppresses ATRA (all trans-retinoic acid) binding, inhibits falling off the receptors, and activates transcription factors like cAMP-response element-binding protein (CREB), Sp1, nuclear transcription factor κ B (NF- κ B), etc. Thus, azadirachtin exerts anti-inflammatory and anti-metastatic responses by a novel pathway that would be beneficial for anti-inflammatory and anti-cancer therapies [38].

Olive

(Biological source: - *Olea europaea*

Family: - Oleaceae)

The chief active components of olive oil include oleic acid, phenolic constituents, and squalene. The main phenolics include hydroxytyrosol, tyrosol, and oleuropein, which occur in highest levels in virgin olive oil and have demonstrated antioxidant activity. Oleic acid, a monounsaturated fatty

acid, and squalene have shown activity in cancer prevention. Olive oil consumption has benefit for colon and breast cancer prevention [39]. Olive fruit extract composed of pentacyclic triterpenes, maslinic acid (73.25%) and oleanolic acid (25.75%). Oleanolic acid shows moderate antiproliferative activity and moderate cytotoxicity at high concentrations on human HT-29 colon cancer cells. Maslinic acid inhibits cell growth without necrotic effects on human HT-29 colon cancer cells. Maslinic acid increases caspase-3-like activity. Maslinic acid generated superoxide anions. Completion of apoptosis by maslinic acid was confirmed by the increase in plasma membrane permeability, and detection of DNA fragmentation. Therefore Maslinic acid shows the chemoprevention of colon cancers [40]. Pinoresinol, the main phenol component of extra virgin olive oil affects the cell viability, which was significantly more pronounced in p53-proficient cells. A P53-proficient cell shows increased apoptosis and G (2)/M arrest. In p53-proficient cells, ataxia telangiectasia mutated (ATM) and its downstream-controlled proteins were upregulated after treatment, with a parallel decrease of cyclin B/cdc2 [41].

Onion

(Biological source: - *Allium cepa* **Family:** -

Amaryllidaceae)

The potential anticarcinogenic action of onions is related to their high content of organosulfur compounds or to their high antioxidant activity, which is principally due to their wide content of flavonoid. However, there are important varietal differences in the composition, concentration, and

beneficial activities of these bioactive compounds, which also result by modalities of cooking. Some studies found that there is a protective role of a moderate frequency of onion consumption against colorectal, laryngeal, and ovarian cancers [42]. Polish white and red onions were subjected to blanching, boiling, frying, and microwaving for different periods of time, and then their bioactive compounds (polyphenols, flavonoids, flavanols, anthocyanins, tannins, and ascorbic acid) and antioxidant activities were determined. It was found that blanching and frying and then microwaving onions did not decrease significantly the amounts of their bioactive compounds and the level of antioxidant activities [43]. Myricetin is one of the principal phytochemicals in onions. Topical treatment with myricetin inhibited repetitive UVB-induced neovascularization in SKH-1 hairless mouse skin. The induction of vascular endothelial growth factor, matrix metalloproteinase (MMP)-9 and MMP-13 expression by chronic UVB irradiation was significantly suppressed by myricetin treatment. Thus myricetin suppresses UVB-induced angiogenesis by regulating PI-3 kinase activity in vivo [44].

Papaya

(Biological source: - *Carica papaya*

Family: - Caricaceae)

In the leaves of *Carica papaya* (CP), components reported to have potential anti-tumor activity include tocopherols, lycopene, flavanoids, and benzyliothiocyanate. CP extract inhibited the proliferative responses of solid tumor cell lines derived from cervical carcinoma (Hela), breast adenocarcinoma (MCF-7),

hepatocellular carcinoma (HepG2), lung adenocarcinoma (PC14), pancreatic epithelioid carcinoma (Panc-1), and mesothelioma (H2452) in a dose-dependent manner. In addition, CP extract inhibits the proliferative responses of haematopoietic cell lines, including T cell lymphoma (Jurkat), plasma cell leukemia (ARH77), Burkitt's lymphoma (Raji), and anaplastic large cell lymphoma (Karpas-299). CP extract enhance the production of anti-tumor cytokines, such as IL-12p40, IL-12p70, IFN- γ and TNF- α . Cytotoxicity of pre-activated PBMC (human peripheral blood mononuclear cells) against K562 was significantly enhanced by treatment of CP extracts at 25:1 and 12.5:1 effector–target ratio (E: T ratio) [45].

Pumpkin

(Biological source: - *Cucurbita maxima*

Family: - Cucurbitaceae)

Preliminary phytochemical study shows the presence of flavonoid, polyphenolics, saponins, protein and carbohydrate in *Cucurbita maxima* extract. Many such compounds are known to possess potent antitumor properties, particularly some proteins and polysaccharide fractions in *Cucurbita maxima* fruits and seeds. The ascitic fluid is essential for tumor growth, since it constitutes a direct nutritional source for tumor cells. *Cucurbita maxima* significantly reduce tumor volume probably by lowering the ascitic nutritional fluid volume. Further, the packed cell volume and the number of viable cells in peritoneum were significantly low. These results indicate either a direct cytotoxic effect on tumor cells or an indirect local effect, which may involve macrophage activation and

vascular permeability inhibition. The increase of life span is seen in animal study. The level of lipid peroxide in liver was significantly reduced to near normal. This reflects the decrease in free radical production and the subsequent reduction in oxidative stress, one of the main risk factors for the disease. Glutathione, a potent inhibitor of neoplastic process plays an important role as an endogenous antioxidant system that is found particularly in high concentration in liver and is known to have key function in the protective process [46].

Pointed gourd

(Biological source: - *Trichosanthes dioica*

Family: - Cucurbitaceae)

Trichosanthes dioica possess potential cytotoxic activity including marked antimitotic effect in plant study. It possesses antioxidant properties due to the presence of phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants [47].

Radish

(Biological source: - *Raphanus sativus*

Family: - Brassicaceae)

Active substance indole-3 carbinole in radish can be use as anti tumor, preventing carcinogenesis against cell line estrogen responsive, serves as immunomodulatory and increase TNF (tumor necrosis factor). The antioxidant properties of radish sprouts in which the glucosinolates glucoraphasatin

(GRH) shows antioxidant activity [48]. Radish leaf inhibits the proliferation of MDA-MB231 through induction of apoptosis and down-regulate Erb B2 signaling and the Akt pathway, making EKRL a potent candidate as new-anti cancer food components [49].

Ridge gourd

(Biological source: - *Luffa acutangula*

Family: - Cucurbitaceae)

Fruits of *L .acutangula* methanolic extract show significant antiproliferative activity on human lung adenocarcinoma epithelial cell line (A-549). VEGF (Vascular endothelial growth factor) is shown to be the most potent angiogenic factor. Studies demonstrated that the expression of VEGF was reduced in a time-dependent manner by *L .acutangula* extract. Matrix metallo-proteinases (MMPs), a family of zinc-dependent endopeptidases, play a crucial role in ECM (extra cellular matrix) degradation associated with tissue repair, cancer cell invasion, metastasis and angiogenesis. Among members of the MMP family, MMP-2 (gelatinase-A) and MMP-9 (gelatinase-B) are particularly up-regulated in malignant tumors. *L .acutangula* extract shows significant inhibition on MMP-2 and MMP-9 indicating the effective role of extract in the prevention of angiogenesis [50]. *L .acutangula* leaf extract have potent hepatoprotective action against carbon tetrachloride induced hepatic damage in rats. Hepatoprotective action is due to free radical scavenging and antioxidant activities which may be due to the presence of flavonoids in the extract [51].

Soyabean

(Biological source: - *Glycine max* **Family:** - Fabaceae)

A 5% dietary supplementation with selectively hydrogenated soybean oil (SHSO) inhibits the growth of prostate cancer by 80% in vivo. SHSO induces apoptosis in prostate cancer cell of rats. DNA fragmentation analysis in vitro further confirms the apoptotic activity of SHSO on the MAT-LyLu prostate cancer cells. The SHSO also shows strong cytotoxicity on human prostate cancer cells (DU145 and PC3) [52]. Glyceollins were major bioactive compounds present in soybean elicited by fungi and shown to have antifungal and anticancer activities. Glyceollins shows a strong reducing power and inhibit lipid peroxidation, with significant scavenging activities of radicals including singlet oxygen, superoxide anion, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and 2,2-diphenyl-1-picrylhydrazyl (DPPH). It was also found that glyceollins significantly suppresses H₂O₂-induced ROS production in HEPA1C7 cells. Therefore, glyceollins deserve further study as natural antioxidants and nutraceuticals [53].

Spinach

(Biological source: - *Spinacia oleracea*

Family: - Amaranthaceae)

Spinach contains the largest amount of sulfoquinovosyl diacylglycerol (SQDG). Spinach had the strongest inhibitory effect on DNA polymerase alpha activity and human cancer cell proliferation. The inhibition of polymerase alpha activity by SQDG may lead to cell growth suppression. Therefore the glycolipids fraction from spinach is potentially a source of food

material for a novel anticancer activity [54]. On oral administration of glyco-glycerolipid fraction from spinach as preliminary medication, colon tumor growth was delayed, and the protein expression level of proliferating cell nuclear antigen (PCNA) was decreases in tumor tissue. It also suppresses sarcoma formation with no adverse reactions in mice [55].

Tomato

(Biological source: - *Solanum lycopersicum*

Family: - Solanaceae)

The combined effects of low concentration of lycopene, a major component in tomato and eicosapentaenoic acid (EPA) may synergistically inhibit the proliferation of human colon cancer HT-29 cells. The inhibitory mechanism was associated with suppression of phosphatidylinositol 3-kinase/Akt signaling pathway. Furthermore, treatment of lycopene and EPA also synergistically blocked the activation of downstream mTOR molecule. Immunocytochemical staining results revealed that lycopene and EPA could also up-regulate the expression of apoptotic proteins such as Bax and Fas ligand to suppress cell survival [56]. Lycopene have potential antitumorigenic activity in skin carcinogenesis assay. 16 % skin carcinoma was observed in DMBA + Croton oil + tomato juice group as compared to DMBA + Croton Oil in which 100 % papillomas were obtained. It seems that topical application of tomato juice prevents the 84 % development of carcinomas. It was observed that DMBA + croton oil is working in initiation and promotion protocol in skin carcinogenesis assay. 24 hours prior applications of tomato extract by i.p. has significantly prevents the

micronucleus formation in bone marrow cells of mice & chromosomal aberration [57]. Tomato extracts causes 50% inhibition of cancer cell growth against the proliferation of the cultured cancer cell line HT-29. The high cytotoxicity for HT-29 cells might be due to the simultaneous presence in the extract of both carotenoids and glyceryl esters of fatty acids [58].

Turnip

(Biological source: - *Brassica rapa* var.

rapa **Family:** - Brassicaceae)

Epidemiological studies suggest that intake of cruciferous vegetables is associated with decreased risks of developing cancers. Turnip contains glucosinolates that are sulfur containing secondary metabolites derived from protein and non-protein amino acids. When plant tissue is damaged, the enzyme myrosinase hydrolyzes glucosinolates into glucose, sulfate, isothiocyanates, nitrile, and thiocyanate. The breakdown products of certain glucosinolates have been shown to protect against lung, colon, liver and stomach cancers. In particular, β -Phenylethyl isothiocyanate, abundant in the peel of turnip shows anti cancer property. HepG2 cells treated with β -phenylethyl isothiocyanate shows a concentration-dependant decrease in cell viability. It is found that isothiocyanate-mediated apoptosis in vivo is associated with the removal of chemically-induced cancer [59].

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A Short Review on Common Plants with their Extraordinary Beneficial Effect on the treatment of Diabetes Mellitus

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Abstract

Herbal medicines derived from medicinal plants are used by about 60% of the world's population. This review focuses on some medicinally rich plants used in the treatment of diabetes worldwide. Diabetes is an important metabolic disorder and at present, approximately 18-20 million people are diabetic in India. Insulin and Oral hypoglycemic agents which are marketed for diabetes are costly than herbal medicine and it is very difficult to bear for urban people. In this paper medicinal plants with proven antidiabetic and related beneficial effects used in treatment of diabetes are discussed. These Plants are *Embllica officinalis*, *Azadirachta indica*, *Allium sativum*, *Annona squamosa*, *Aegle marmelos*, *Elephantopus scaber*, *Musa Paradisiaca*, *Andrographis Paniculata*, *Mangifera indica*.

Key words: Diabetes Mellitus, Herbal medicine, hypoglycemic activity

Introduction

Diabetes mellitus is a systemic metabolic disease by the abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin.

This disease characterized by hyperglycemia, hyperlipidemia, hyperaminoacidemia and hypoinsulinaemia it leads to decrease in both insulin secretion and insulin action. Diabetes mellitus is mainly two types, Insulin dependent diabetes mellitus (IDDM) and Non insulin dependent diabetes mellitus (NIDDM) but another type is gestational diabetes. It is frequently associated with the development of micro and macro vascular diseases which include neuropathy, nephropathy, cardiovascular and cerebrovascular diseases

[1] Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. It is estimated that more than 300 million people in the world will have diabetes by the year 2025. At presently, approximately 18-20 million people are diabetic in India, and it is projected that by 2025 there will be 20-60 million diabetics in India, and it will have the second largest number of diabetics in the world [2]. Plants and human have strong relationship from prehistoric age either for Food or Home or drug. Ancient man tried to restore to health from diseases by plant's parts growing around him. The different medicinal characters of plants were learnt by trial and error method. Siddha, Unani and Ayurveda

are very well traditional practice in India and Nepal for more than 2000 years ago. Many European countries and organizations like National Institute of Health (NIH), USA, and World Health Organization (WHO) have recognized ayurveda as an alternative and complementary medicine. Science has deep knowledge of physical and biological structure of human body; whereas traditional knowledge systems have a holistic knowledge of the physical and spiritual fields that pervade nature [3]. Modern science has deep knowledge of physical and biological structure of human body, whereas Ayurveda system deals with the role of mind and consciousness in health and disease states. Recently Ayurveda plays an important role as alternative medicine due to fewer side effects than oral hypoglycemic agents and low cost. The ethnobotanical information reports about 1000 plants that may possess antidiabetic potential [4]. The active principles present in medicinal plants have been reported to possess pancreatic beta cells re-generating, insulin releasing and fighting the problem of insulin resistance [5]. This review article enumerates some medicinal plants possessing hypoglycemic properties and elucidating their family, common name, parts used, chemistry and other pharmacological activity of some plants such as *Emblica officinalis*, *Azadirachta indica*, *Allium sativum*, *Annona squamosa*, *Aegle marmelos*, *Elephantopus scaber*, *Musa Paradisiaca*, *Andrographis Paniculata*, *Mangifera indica*.

Important Medicinal Plants having Antidiabetic Potential:

Emblica officinalis (Amlaki)

Family- *Euphorbiaceae*.

Common Name- Dhatriphala, Amla, Amaliki, Amalakan, Sripthalam, Vayastha (Sanskrit) An Mole (Chinese), Popok Melaka (Malaysian), Mirabolano emblico (Portuguese), Phyllanthus Emblica or Indian gooseberry.

Parts used- Dried fruits, Fresh fruit, seed, leaves, root, bark, flowers.

Geological source- It grows in tropical and subtropical regions including Pakistan, Uzbekistan, Srilanka, South East Asia, China and Malaysia.

Chemistry

Emblica officinalis contains tannins, alkaloids, phenolic compounds, amino acids and carbohydrates. Its fruit juice contains the highest vitamin C (478.56 mg/100 mL). The fruit when blended with other fruits boosted their nutritional quality in terms of vitamin C content [6]. Amla contains gallic acid, ellagic acid, 1-O-galloyl-beta-D-glucose, 3,6-di-O-galloyl-Dglucose, chebulinic acid, quercetin, chebulagic acid, corilagin, 1,6-di-O-galloyl beta D glucose, 3 Ethylgallic acid (3 ethoxy 4,5 dihydroxy benzoic acid) and isostrictiniin [7]. Phyllanthus emblica also contains flavonoids, kaempferol 3 O alpha L (6" methyl) rhamnopyranoside and kaempferol 3 O alpha L (6" ethyl) rhamnopyranoside [8]. A new acylated apigenin glucoside (apigenin 7 O (6" butyryl) beta glucopyranoside) was isolated from the methanolic extract of the leaves of Phyllanthus emblica together with the known compounds; gallic acid, methyl gallate, 1,2,3,4,6-penta-O-galloylglucose and luteolin-4'-Oneohesperidoside were also reported [9].

Pharmacological Study on Diabetes

Oral administration of the extract (100mg/kg body weight) can reduce blood sugar level in normal as well as alloxan (120mg/kg body weight) induce diabetic rats within 4 hours. It also has power to delay the development of diabetic cataract in rats [10]. It also has important inhibitory activity on Aldose reductase (AR) enzyme which has an ability to produce post diabetic complications [11].

Pharmacology

It has its beneficial role in cancer, diabetes, liver disease, heart trouble, ulcer, anemia. Similarly, it has application as antioxidant, immunomodulatory, antipyretic, analgesic, cytoprotective, antitussive and gastroprotective. Additionally, it is useful in memory enhancing, ophthalmic disorders and lowering cholesterol level. It is also helpful in neutralizing snake venom and as an antimicrobial [12].

Herbal Dosage form & Dose

It is used as Amalaki capsules as dose of 1 capsule/ twice a day before meal. It is often used in the form of Triphla churna.

Azadirachta indica (Neem)

Family Meliaceae

Common Name-

Neem, Nim (Bengali, Hindi, Assamese), Azad-darakhul-

hind (Arabic), Baypay (Malaysia), Crackjack, Chinaberry (English), Grossblättiger zedrach (French)

Parts used - Leaf, Bark, Flower, Fruit, Twig, Gum, Seed, pulp.

Geological source

It is obtained in India, Burma, Pakistan, Sri Lanka and Bangladesh growing

in tropical and semi-tropical regions. Neem tree is the official tree of the Sindh Province and is very common in all cities of Sindh. Neem trees also grow in islands in the southern part of Iran where it is called "Cherish" or Azad derakht in Persian.

Chemistry

More than 135 compounds have been isolated from different parts of Neem. The compounds have been divided into two major classes: isoprenoids and others. The isoprenoids include diterpenoids and triterpenoids containing protomeliacins, limonoids, azadirone and its derivatives, gedunin and its derivatives, vilasinin type of compounds and Csecomeliacins such as nimbin, salanin and azadirachtin. The nonisoprenoids include proteins (amino acids) and carbohydrates (polysaccharides), sulphurous compounds, polyphenolics such as flavonoids and their glycosides, dihydrochalcone, coumarin and tannins, aliphatic compounds, etc. Main constituents are Nimbidin (Anti-inflammatory, Antiarthritic, Antipyretic, Hypoglycaemic, Antigastric, ulcer, Spermicidal, Antifungal, Antibacterial, Diuretic), Sodium nimbidate (Anti-inflammatory), Nimbin (Spermicidal), Nimbolide, Gedunin, Azadirachtin, Mahmoodin, Gallic acid, (-) epicatechin and catechin, Margolone, margolonone and isomargolonone, NB-II peptidoglycan (Immunomodulatory) [13].

Pharmacological Study on Diabetes

Aqueous extract of neem leaves significantly decreases blood sugar level and prevents adrenaline as well as glucose-induced hyperglycaemia. The aqueous leaf extract when orally fed, also produces

hypoglycaemia in normal rats and decreased blood glucose levels in experimentally-induced diabetes in rats. Aqueous leaf extract also reduces hyperglycaemia in streptozotocin diabetes and the effect is possibly due to presence of a flavonoid, quercetin . A significant hypoglycaemic effect was also observed by feeding neem oil to fasting rabbits²⁴. Recently, hypoglycaemic effect was observed with leaf extract and seed oil, in normal as well as alloxan-induced diabetic rabbits [14].

Pharmacology

Anti-inflammatory, antipyretic and analgesic activities, Immunostimulant activity, Antiulcer effect, Antifertility effect, Antimalarial activity, Antifungal activity, Antibacterial activity, Antiviral activity, Anticarcinogenic activity, Hepatoprotective activity, Effect on central nervous system, Antioxidant activity [15].

Herbal Dosage form & Dose

Each capsule contains freeze dried powder of Neem 200 mg. Adult dose 1-2 capsules in morning and evening after meals. Dose of Children - (5-10 years) 1 capsule morning & evening with milk.

Allium sativum(Garlic)

Family Liliaceae

Common Name- Garlic (eng), Lasan (Guj), Lasun (Hindi), Lashuna (Sanskrit).

Parts used - Ripe Bulbs.

Geological Source- It grows in Central Asia, Southern Europe, USA, and India.

Chemistry

The main chemical constituent of intact garlic is the amino acid alliin, an alkyl derivative of cysteine alkyl sulfoxide, which may varies from 0.2 to 2.0% fresh weight [16] it contains a wealth of sulphur compounds; most important for the taste is Allicin , which is produced enzymatically from allin. It also contains phosphorus, iron & copper. Volatile oil of the drug is the chief active constituent, and contains allyl propyl disulphide, diallyl disulphide, alliin and allicin. It also contain 65% water, 28% carbohydrate, 2.3% organosulphur compound, 2% proteins, 12% free amino acid(mainly arginine) , 1.5% fiber, 0.15% lipids, 0.08% phytic acid, 0.07% saponins [17].

Pharmacological Study on Diabetes

S-allyl cystein sulfoxide (SACS), the precursor of Allicin and garlic oil, is a sulfur containing amino acid, which controlled lipid peroxidation better than glibenclamide and insulin. It also improved diabetic conditions. SACS also stimulated in vitro insulin secretion from beta cells isolated from normal rats [18].

Pharmacology

It is use in the treatment of sickle cell anemia, antimicrobial, antithrombotic, hypolipidemic, Antihypertensive, antiarthritic, hypoglycemic, antioxidant activity and antitumor activity .

Herbal Dosage form & Dose

Juice extract of garlic is used. Juice extract- 50 ml / daily.

***Annona squamosa* Linn (Sugar apple)**

Family- Annonaceae

Common Name- Custard apple, sugar apple, sweet après in english, & sharifa in hindi, Ata in Bengali & sitaphalam in telugu in india & corossolier & cailleux, pommier cannelle in French.

Parts used –Fruits, Leaves, Root, and Stems.

Geological Source- It is commonly found in India & cultivated in Thailand & originates from the West Indies & South America [19].

Chemistry

The plant is reported to contain glycoside, alkaloids, saponins, flavonoids, tannins, carbohydrates, proteins, phenolic compounds, phytosterols, amino acids .The various chemical constituents isolated from leaves, stems and roots of the plant including anonaine, aporphine, coryline, isocorydine, norcorydine, glaucine. Leaves contains 4-(2-nitro-ethyl 1)-1-6-((6-o- β -D-xylopyranosyl- β -D-glucopyranosyl)-oxy) benzene, Anonaine, Benzyltetrahydroisoquinoline, Borneol, Camphene, Camphor, car-3-ene, Carvone, β -Caryphyllene, Eugenol, Farnesol, Geraniol, 16-Hetriacontanone, Hexacontanol, Higemamine, Isocorydine, Limonine, Linalool acetate, Menthone, Methyl anthranilate, Methylsalicylate, Methylheptenone, p-(hydroxybenzyl)-6,7-(2-hydroxy,4-hydro)isoquinoline, n-Octacosanol, a-Pinene, b-Pinene, Rutin, Stigmasterol, β -Sitosterol, hymol and n-Triacontanol. Alkaloids, proteins & amino acids are absent in the leaf extract [20].

Pharmacological Study on Diabetes

The antihyperglycemic activity of the Aqueous. Extract of *Annona squamosa* roots was comparable with glibenclamide, a standard hypoglycaemic drug [21] . The ethanolic extract of *Annona squamosa* Linn leaves posses considerable hypoglycemic activity in normal rats. The dose of 350 mg/kg body weight reduced the fasting blood glucose level by 6.0% within 1 h, whereas, the peak blood glucose at 1 h during glucose tolerance test was reduced by 17.1% in normal rats. Treatment of alloxan-induced diabetic rabbits for 15 days with a dose of 350 mg/kg of extract reduces fasting blood glucose by 52.7 % and urine sugar by 75%. The dose of 350 mg/kg body weight of ethanolic extract in 10-day treatment of a group of STZ-diabetic rats produced 73.3% fall in FBG level and no sugar was observed in fasting urine. [22]. An aqueous extract of *A. squamosa* leaves found to lower considerable fasting plasma glucose level in streptozotocin-nicotinamide induced type 2 diabetic rats. The findings of the study support the antidiabetic claims of *A. squamosa* [23].

Pharmacology

Annona squamosa Linn is used as an antioxidant, Antibacterial activity, Antidiabetics, hepatoprotective, Antihyperlipidemic Activity, Antimicrobial activity, Antithyroidic activity , Molluscicidal activity, Antiplasmodial activity, Vasorelaxant activity, Anti-platelet activity, Anti-inflammatory activity , Antifertility activity, Antiviral activity, Anthelmintic activity, Chemopreventive & Antilipidperoxidative, cytotoxicactivity,

genotoxicity, antitumour activity, antilice agent, Insecticidal Activity, Mosquitocidal activity, Pesticidal activity [24].

Herbal Dosage form & Dose

Annona squamosa Powder and Annona squamosa extract and oil is used.

Aegle marmelos (Bael)

Family- Rutaceae

Common Name-

Bel, Beli (Hindi & Bengali) Shivaphala (Sanskrit), Vilvama (Tamil), Marredy (Malayalam).

Parts used – Unripe or half ripe fruits

Geological Source- The Bael tree has its origin from Eastern Ghats and central India. It is also found in tropical and a subtropical region of India. Bael is found growing along foothills of Himalayas, Uttaranchal, Jharkhand, Madhya Pradesh, East coast.

Chemistry

The chief constituent of the drug is marmelosin (0.5%) which is a furocoumarin. Other coumarins are marmesin, psoralin, umbelliferone. The drug also contains carbohydrate, protein, volatile oil & tannins. The pulp also contains good amount of vitamin A & C. Two alkaloids, O-methylhalfordinol & isopentylhalfordinol have been isolated from fruits. Its leaves contained γ -sitosterol, aegelin, lupeol, rutin, marmesinin, β -sitosterol, flavones, glycoside, O-isopentylhalfordiol, marmeline and phenylethyl cinnamamides. Leaves of bael contain alkaloids like halfordino, ethylcinnamamide, phenylcinnamide, anhydromarmeline, aegelinosides A and B. Phenylpropenoids are included

hydroxycoumarins, phenylpropenes and lignans. It also contains tannins like skimmianine and contains small amount of carotenoids [25].

Pharmacological Study on Diabetes

A significant decrease in liver glycogen of diabetic rats is reversed to almost the normal level by the leaf extract and it also decreases the blood urea and serum cholesterol. A similar effect is seen with insulin treatment and the results indicate that the active principle in *A. marmelos* leaf extract has similar hypoglycemic activity to insulin treatment [26]. The hypoglycemic effect of water extract of the fruits of *Aegle marmelos* was examined in streptozotocin induced diabetic wistar rats. The effect of the extract at a dose of 250 mg/kg-1 was more effective than glibenclamide [27].

Pharmacology

Pharmacological activity of leaves-

Contractile activity, Anti-microfilarial activity, Analgesic Activity, Anti-inflammatory, Antipyretic, Analgesic activity, Anti-ulcer activity, Anticonvulsant activity, Antidepressant, Anxiolytic activity, Antifertility activity, Antifungal activity, Hepatoprotective activity, Radioprotective activity, Hypolipidemic activity, Immunomodulatory activity. Pharmacological activity of fruits- Hypoglycemic Activity. Pharmacological activity of Seed- Hypoglycemic activity, Antifungal activity.

Herbal Dosage form & Dose

Aegle marmelos is used as aqueous decoction & aqueous leaf extract. Aqueous

decoction- 1 ml/ 100 mg, Aqueous leaf extract- 1 gm/ kg/BW.

Elephantopus scaber(*Elephant's foot*)

Family- Asteraceae

Common Name-Prickly Leaved, Elephant's Foot, Hindi- Samdudri, Bengali-Hasti pod.

Parts use- Whole plant, Leaves, Root.

Geological source- It is Native to East Tropical Africa, West-Central Tropical Africa, South Tropical Africa, China, Japan, Indian Subcontinent(Central Eastern Ghats region), Malaysia, Australia, Philippines.

Chemistry

Phytochemical constituents of *Elephantopus scaber* L. have been reported as sesquiterpene lactones, elephantopin and scabertopin [28] epofriedelinol, lupeol and stigmasterol. It is also contained Terpenoids and 2,6,23 –trienolide compounds which is a potential candidate of diabetes [29].

Pharmacological Study on Diabetes

Hypoglycemic effect of *E.Scaber* was introduced by alloxan in male wistar albino rats. Oral administration of aqueous extract of *Elephantopus Scaber* leaves (300mg/kg body weight) and roots (300mg/kg body weight) for 84 days significantly reduced serum glucose, glycosylated hemoglobin and activity of glucose-6-phosphatase but increase serum insulin, liver and skeletal muscle glycogen activity [30]. The antidiabetic property of plants shows their mechanisms by improving insulin

sensitivity, augmenting glucosedependent insulin secretion and stimulating the regeneration of islets of langerhans in pancreas of STZ- induced diabetic rats [31].

Pharmacology

The leaves of the plant were known to be used for fever, elimination of bladder stone, diuretic, bronchitis, small pox, diarrhea, stomach pain, liver tonic and as a brain tonic (Reico .,78-79) anti inflammatory and antitumor activity [32]. The roots of *Elephantopus scaber* were broadly used as an antipyretic, cardio tonic, haemorrhoids and diuretic. Leaves of *Elephantopus Scaber* shows antimicrobial activity more than root. The aqueous extract of leaves is applied externally to treat eczema and ulcers [33]. The methanolic extract of root of *E. scaber* shows antioxidant activity and antihepatotoxic activity [34].

Herbal Dosage form & Dose

Elephantopus Scaber extract is use as food for supplement (Thiland).

Musa Paradisiacal (Banana)

Family- Musaceae.

Common Name- Banana, Pisang.

Parts used-Seed, fruit. Leaves.

Geological Source- It is mostly abundant in Asian, IndoMalaysian and Australian tropics and is now widely found throughout the tropical and subtropical countries. India, Bangladesh, Philippines, China, Ecuador, Brazil, Indonesia, Mexico, Costa Rica, Colombia, Thailand [35].

Chemistry- Serotonin, nor-epinephrine, tryptophan, indole compounds, tannin, starch, iron, crystallisable and non-crystallisable sugars, Pectin, vitamin C, B-vitamins, albuminoids, fats, mineral salts have been found in the fruit pulp of *M.*

paradisiacal [36]. Several flavonoids and related compounds (Leucocyanidin, quercetin and its 3-Ogalactoside, 3-O-glucoside, and 3-O-rhamnosyl glucoside) were isolated from the unripe pulp [37] [38]. Cellulose, hemicelluloses, arginine, aspartic acid, glutamic acid, leucine, valine, phenylalanine and threonine have been isolated from pulp and peel of *M. paradisiaca* [39] [40]. Hemiterpenoid glucoside, syringin, roseoside, benzyl alcohol glucoside, (24R)-4 α ,14 α ,24-trimethyl-Sacholesta-8,25(27)-dien-3 β -ol have been isolated from flower of *M. paradisiaca* [41] [42] (Duita et al., 1983; Martin et al., 2000). Acyl steryl glycosides such as sitoindoside-I-IV, and steryl glycosides such as sitosterol gentiobioside, sitosterol myo-inositol- β -D-glucoside have been isolated from fruits of *M. paradisiaca* [43]. It also contained L-tryptophan 5-Hydroxytryptamine, Syringin, 7, 8-dihydroxy-3-methyl isochroman-4-one.

Pharmacological Study on Diabetes

The green fruit of *M. paradisiaca* has been reported to have hypoglycemic effect due to stimulation of insulin production and glucose utilization [44]. Its high potassium (K) and sodium (Na) content has been correlated with the glycemic effect [45]. Fibers from *M. paradisiaca* fruit increased glycogenesis in the liver and lowered fasting blood glucose [46]. Antihyperglycemic effect of the hydromethanolic extract of *M. paradisiaca* root has been found significant [47] [48]. *M. paradisiaca* stem juice showed hyperglycemic activity.

Pharmacology

Antidiarrhoeal activity, Antiulcer activity, Antimicrobial activity, Hypocholesterolaemic activity,

Antihypertensive activity, Effect in atherosclerosis, Antioxidant activity, Diuretic activity, Wound healing activity, Anti-allergic activity, Antimalarial activity, Anti-snake venom activity, Mutagenicity [49].

Herbal Dosage form & Dose

Musa Paradisiacal extract and banana leaves extract are used.

Andrographis paniculata Nees. I (Kalmegh)

Family: Rutaceae

Common name- Kalmegh (Hindi), chuan xin Man (Chinese).

Parts used- Whole plant

Geographical source- It is an herbaceous plant native to India, Sri Lanka and widely cultivated in southern Asia.

Chemistry- *A. paniculata* contains diterpenes, lactones, and flavonoids. Flavonoids mainly exist in the root, but have also been isolated from the leaves. The aerial parts contain alkanes, ketones, and aldehydes. The leaves contained two bitter principles – andrographolide and a compound named kalmeghin. Four lactones – chuanxinlian A (deoxyandrographolide), B (andrographolide), C (neoandrographolide) and D (14-deoxy-11, 12-didehydroandrographolide) – were isolated from the aerial parts [50]. Bis-andrographolides A, B, C, and D have been isolated from aerial parts. Two new flavonoids identified as 5,7,2',3'-tetramethoxyflavanone and 5-hydroxy-7,2',3'-trimethoxyflavone were isolated from the whole plant (Koteswara., 2004), while 12 new flavonoids and 14 diterpenoids have been reported from the aerial parts [51]. Two new flavonoid glycosides and a new

diterpenoid (andrographic acid) were recently reported.

Pharmacological Study on Diabetes-

It is reported that hypoglycemic activity of *Andrographis paniculata*. A significant decrease in blood glucose levels was observed on glucose tolerance test as compared to the untreated group. Oral administration of *andrographis* significantly increases the activity of SOD and Catalase. Also decreases blood glucose levels due to its antioxidant properties [52]. The ethanol extract of *A. paniculata* possesses antidiabetic property and may be attributed at least in part to increase glucose metabolism. Its hypotriglyceridemic effect is also beneficial in the diabetic state [53].

Pharmacology

It shows Hepatoprotective activity, Antimicrobial, Antiparasitic activity, Antioxidant, anti-inflammatory activity, Antihyperglycemic and Hypoglycemic Effect Dysentery/Gastroenteritis.

Herbal Dosage form & Dose

Dried herb: 1.5 to 5 g/day.

Tea: 1/2 to 1 tsp dried herb in 8 oz hot water, steep 30 minutes, take 4 oz three times daily.

Tincture: (1:5, 30% alcohol): 20 to 60 gtt (1-3 mL) three times daily.

Standardized Tablets: 100-mg tablets containing 5 mg andrographolide and deoxyandrographolide, four tablets three times daily.

Kan Jang (SHA-10) is a proprietary extract derived from *Andrographis paniculata* and

Eleutherococcus senticosus, one 350 mg tablet twice a day.

***Mangifera indica* (Aam)**

Family- Anacardiaceae

Common Name- Aam (Hindi, Bengali), Mango (English), An Lo Kuo (China).

Parts used- Bark, root, Leaves.

Geological Source- *M. indica* resides in most tropical biotopes in India, Southeast Asia, Malaysia, Himalayan regions, Sri Lanka, Africa, America and Australia, Mexico from the Philippines and the West Indies.

Chemistry-

The bark contains protocatechic acid, catechin, mangiferin, alanine, glycine, γ -amino-butyric acid, kinic acid, shikimic acid and the tetracyclic triterpenoids cycloart-24-en-3 β ,26-diol, 3-ketodammar-24(E)-en-20S,26-diol. C-24 epimers of cycloart-25-en-3 β ,24,27-triol and cycloartan-3 β ,24,27-triol [54]. The natural C-glucoside xanthone mangiferin has been reported in various parts of *M. indica*: leaves, fruits, stem bark, heartwood and root.

Pharmacological Study on Diabetes-

It is already investigated the effects of mangiferin on hyperglycaemia, atherogenicity and oxidative damage to cardiac and renal tissues in streptozotocin-induced diabetic rats (STZ destroys pancreatic β cells and causes persistent hyperglycaemia; 55 mg/kg body weight i.v.); after 30 days, diabetic rats were administered mangiferin or insulin (positive control) daily for 28 days. These studies show that mangiferin (10 and 20 mg/kg, i.p.) exhibits potent antidiabetic, antihyperlipidemic, antiatherogenic and

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Pharmaceutical Excipients from Natural Sources

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Abstract

The present study, reviews and summarizes the natural pharmaceutical excipients, its uses, safety concerns, comparison with synthetic ones and present status in pharmaceutical industry. Natural pharmaceutical excipients are inactive ingredients which are used to formulate pharmaceutical dosage forms and these are obtained from different natural sources. Extensive use of synthetic excipients has also revealed their drawback and toxic effects. Today pharmaceutical industries slowly making more out of natural excipients owing to their certain advantages over their synthetic counterparts. Various safety parameters and standards have also been specified. Today the traditional concept of the excipients as the one other than the active substance has undergone a substantial evolution from an inert and inexpensive vehicle to an essential and sophisticated constituent of the formulation. Global excipients market is expected to grow rapidly with the emerging trends in the pharmaceutical industry and to be specific natural excipients are not far behind. Further more study and research work is needed especially on compatibility and safety matters which may enable it to lead in the use of modern pharmaceutical dosage forms and thereby making economically more important in future.

Key words: Natural pharmaceutical excipients, dosage forms, synthetic excipients, polymer, gum

INTRODUCTION

Pharmaceutical excipients are the substances which are inactive pharmaceutical ingredients (IPI) other than the API.

These are used as a medium for giving a medicament with simply the functions of an inert support of the active principles [1]. The variability of active compounds, excipients and process are obvious components for the product variability. Nature has provided us a wide variety of materials to improve the drug delivery systems. In recent years, plant derived polymers have evoked tremendous interest due to their diverse pharmaceutical applications such as diluent, binder, disintegrant in tablets, thickeners in oral liquids, protective colloids in suspensions, gelling agents in gels and bases in

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suppository. Today we have several excipients of plant origin like starch, agar, alignates, guar gum, xanthan gum, gelatin, pectin, acacia, tragacanth, cellulose etc. The natural materials have advantages like they are chemically inert, non toxic, less expensive, bio-degradable, widely available, biocompatible, public acceptance etc [2]. Natural polysaccharides are extensively used for the development of solid dosage forms. The polymers of mono- saccharides (sugar) are inexpensive and available in variety of structures with a variety of properties. The application of these excipients in pharmaceutical industry as binding agents, fillers, disintegrates, sustaining agents, thickening agents, gelling agents, bases in suppositories, coating materials, stabilizers etc. Excipients are sometimes used to bulking up formulations that contain very potent active ingredients. Development of excipients from natural sources requires complete characterization of the substance is needed [3]. This review gives an insight of nature based pharmaceutical excipients.

NEED FOR NEW EXCIPIENTS

Even though there are a large number of excipients available for formulation development, there are still some lacunae in the range of presently available excipients. Some drugs show incompatibilities with many of the current range of excipients. Also there are not many excipients that allow faster manufacturing of formulations, particularly in case of tablets, there is a need for new materials possessing better compressibility at very high compression speeds [4]. There is a need for new excipients to overcome the obvious disadvantages with the currently available

materials, like magnesium stearate is a most commonly used excellent lubricant, but renders the tablet hydrophobic; hence a readily soluble lubricant as effective as magnesium stearate, is cheerfully welcomed by the pharmaceutical industry.

CHARACTERISTICS OF PHARMACEUTICAL EXCIPIENTS : [5]

Ideal quality of pharmaceutical excipients are as follows:

- Must be physiological inert.
- Must be acceptably Low.
- Must be physically & chemically stable by themselves & in combination with the drug & other dosage components.
- Must be non-toxic & acceptable to the regulatory agencies in all countries where the product is to be marketed.
- Must be commercially available in an acceptable grade in all countries where the product is to be manufactured.
- Must be color – compatible
- Must have no deleterious effect on the bioavailability of the drug(s) in the product.
- Must be free from any unacceptable microbiologic load.
- Must not in any group of population for example sucrose in diabetic patients & sodium in hypertensive patients.

CLASSIFICATION OF NATURAL EXCIPIENTS: BASED ON THEIR ORIGIN:

Based on their origin natural excipients are broadly classified into three categories:

1. Plant origin excipients : Source is plant origin. Examples include Acacia, Starch, Pectin.
2. Mineral origin excipients : Source is mineral origin examples include bentonite, veegum etc.
3. Animal origin excipients : Source is Animal origin. Examples include Gelatin, Egg yolk, wool fat.
4. Microbial origin (fungi and bacteria): Source is fungi or bacteria. glycan, pullula, dextran, xanthan, gellan.

CLASSIFICATION OF NATURAL EXCIPIENTS BASED ON THEIR FUNCTIONS :

On the basic of their function, which excipients play in the finished dosage form, they may be

classified and their details are as follows :

1. DILUENT : These are also known as fillers. They are added to table formulations to increase the bulk of the tabet when the drug dose is inadequate to produce the bulk. Natural diluents includes:

i) Starch : Starch consists of polysaccharide granules obtained from the grains of maize (zea mays), rice (oryza gativa) or weat (triticum aestium), belonging to family Graminea or from the tubers of potato (solanum tuberosum), family solanaceae. Various directly compressible starches are now available commercially for example Sta-Rx 1500, which is free-flowing directly compressible starch and it may used as diluent, binder and/or disintegrating agent. Sta-Rx 1500 contains about 10% moisture &

has got self lubricating properties. [6] Two hydrolyzed starches Emdex and celutab which are basically 90-92% dextrose & directly compressible. These materials contain about 8% - 10% moisture [7]

(ii) Bentonite : Bentonite is a native clay basically it is colloidal hydrated aluminum silicate. It is insoluble in water, swells to approximately twele films its volume upon addition water. It is use as a diluent for wet granulation.[8]

2. BINDERS & ADHESIVE (GRANULATORS) :

These are the substances used to hold powders together toform granules or sometimes promote cohesive compacts for holding the compressed tablet ultimately together after comperession, during handling and shipping.

(i) Natural gums : Natural gums which are used as granulating agents or binders are :

(a) Acacia : Gum acacia/Gum arabic is the dried gummy exudation obtained from the stem & branches of *Acacia arabica* belonging to family leguminosae. It is soluble in water & insoluble in alcohol. It can be used as binder in the form of solution ranging from 10-25% concentration alone or in combination with tragacanth.[9]

(b) Tragacanth : It is the dried gummy exudation obtained by incision from stems & branchesof *Astragulus gummifer* belonging to family leguminosae. Gum tragacanth is a branched, heterogeneous, and anionic carbohydrate which consists of two major fractions: tragacanthin (water-soluble) and bassorin (water-swellaable) Mucilage of tragacanth (10% - 25%) is used as a binding agent in the tablets [10,11]

(ii) Gelatin : Gelatin is a natural protein and is sometimes used in combination with acacia. It

is a more consistent material to prepare in solution form and forms tablets equally as hard as

tragacanth. Also the basic empty capsule shells are made from a mixture of gelatin, sugar & water are clear, colorless, essentially tasteless [12]

(iii) Starch Paste : It is one of the most commonly used granulating agent. It is used in the concentration range of 5-15%. It is prepared by dispersing starch into water, which is then heated for some prescribed time. During the heating, the starch undergoes hydrolysis to dextrin & to glucose. Starch paste is translucent rather than clear and produces cohesive tablets and when properly formulated, it readily disintegrates [13]

3. DISINTEGRANTS: Disintegrants expand and dissolve when wet causing the tablet to break apart in the digestive tract, releasing the active ingredients for absorption. They ensure that when the tablet is in contact with water, it rapidly breaks down into smaller fragments, facilitating dissolution.

Disintegrants act by three mechanisms

- By swelling (Busters) for example Starch
- By improving penetration of aqueous liquids (Wetting agents) for example clays
- By liberation of gas from an effervescent base for example citric acid.

Naturally occurring disintegrants includes:

(i) Starch : Starch USP & various starch derivatives are the most common disintegrating

agent, having lowest cost. Starch is typically used in a concentration range of 5%

to 20% of tablet weight. Some modified starches are Primogel and Explotab. Various pre-gelatinized starches usually in a 5% concentration are also employed as disintegrants [14]

(ii) Gellan Gum: It is a exocellular heteropolysaccharide, anionic microbial polysaccharide, secreted from *Sphingomonas elodea* , produced by fermentation, having high gel strength, high clarity, excellent film forming capacity. It is used as a disintegrant for tablets & capsules [15].

(iii) Guar Gum: Guar gum is the powder of the endosperm of the seeds of *Cyamopsis tetragonolobus*, belonging to family leguminosae. It is colourless or pale yellowish-white coloured powder with characteristic odour & gummy taste. Its 1% mucilage is similar in viscosity as that of mucilage of acacia & 3% mucilage is similar to mucilage of tragacanth.

It has 5 to 8 times thickening power than starch. It is used as a protective colloid, a binding agent and a disintegrating agent too. [16]

(iv) Clays : Clays such as veegum Hv and bentonite have been used as disintegrants at about a 10% level. Such use of these materials is limited unless the tablets are coloured, since the clays produce an off-which appearance. The clays are typically less effective as disintegrants than some of the newer modified polymers & starches,

which can increase in volume in the presence of water by 200% to 500%. [17]

(v) *Locust bean gum*: It is also known as Carob bean gum, extracted from the endosperm of the

leguminous plants, Carob tree (*Ceratonia siliqua*) mostly grows in Spain and in other Mediterranean countries [18,19]

4. LUBRICANTS, GLIDANTS AND ANTIADHERANTS : Lubricants are intended to reduce the friction during tablet ejection between the walls of the tables and the walls of the die cavity in which the tablet is formed. Glidants promote flow of the tablet granulations as powder materials by reducing friction between the particles. Antiadherents are used to reduce sticking or adhesion or any of the tablet granules or power to the face of the punches or to the die wall. Examples of natural Lubricants/Glidants/Antiadherants are :

(i) Vegetable oil : Vegetable oils are used as internal lubricants that is they are mixed with the

dry powder prior to moistening with granulating fluid (Self Lubricating). Among vegetable based fatty acid Lubricants Tristar 149 is widely used Lubricants. It is a fine white powder composed of 90% palmitic & stearic acid. It is used in wet granulation, dry granulation and in direct compression in the form of Lubricants. It is useful for chewable tablets, makes hard shiny tablets without impending dissolution. Less sensitive to overblending than the metal stearates not reactive with acid.[20]

(ii) Starch : Starch has been reported to possess some Lubricating features being a natural

Lubricants it does not damage or the finished dosage form.

5. COLORS, FLAVORS & SWEETENARS: Natural colors flavors/sweetness which are used in pharmaceutical dosage forms includes: [21-25]

1. Natural colors for example

(i) Cochineal, carotene, chlorophyll, curcumin, *Theobroma cacao*

(ii) Saffron : It consist of dried stigmas & upper parts of style of plant known as *Crocus sativus* belonging to family Iridaceae. It has got strong, characteristic & aromatic odour too. It

is used as a coloring (food dye) & flavoring agent.

(iii) Directly compressible dried honey: It is golden yellow, granular, free-flowing power that maintains the flavor enhancing, taste masking of undesirable flavors. Examples of Natural flavors include clove, eucalyptus, lemon, mint, orange, winter green, jasmine, lavender, rose etc.

Taste and flavors are matched like this way also:

Alkaline Taste: Mint, Vanilla

Acid (some) Taste: Lemon, Orange, Onion, Liquorice, Raspberry,

Bitter Taste: Anise, mint, fennel, cherry

Salty Taste: Citrus flavors, Raspberry, melon.

Sweet Taste: Vanilla, Honey

Naturally occurring Sweetening agent include Glycerrhizin, Neohesperidin, Honey, Maple syrup etc. On the contrary, Sodium Saccharine (Artificial Sweetner) has been found to be carcinogenic.

Aspartame, as artificial sweetner, has been blamed for hyperactivity in children.

6. NATURAL PRESERVATIVES : A preservative is a naturally occurring or synthetically produced substance that is added to products such as foods, pharmaceuticals etc. to prevent decomposition by microbial growth or by undesirable chemical changes. Naturally occurring substances such as rosemary extract, hops, salt, sugar, vinegar, alcohol, diatomaceous earth Grapefruit Seed Extract,, Bee Propolis and castor oil are also used as

traditional preservatives. [26] .Sabinsa Corporation's SabiLize®, a natural preservative composition has been granted US Patent for its application as an anti-oxidant and anti-microbial preservative component for pre-pasteurised cosmetic formulations. At 0.5 per cent in creams was found to be significantly effective against *Staphylococcus aureus*, *Escherichia coli*, as well as *Candida albicans* and *Aspergillus niger* [27].

TABLE 1: PHARMACEUTICAL APPLICATION OR USES OF SOME MORE NATURAL EXCIPIENTS

Common Name	Pharmaceutical Applications
Agar	Suspending and emulsifying agent in suppositories,laxative surgical lubricant [28]
Albizia gum	Tablet binder [29]
Aloe mucilage	Gelling Agent , Sustained release agent [30] agent [30]
Bavchi mucilage	Suspending agent[31]
Cassia tora	Binding agent [32].
Gum acacia	Suspending agent, binder in Tablets, demulcent and emollient in cosmetics [33].
Gum tragacanth	Suspending agent, binder in Tablets, demulcent [34]
Khaya gum	Binding agent.[35]
Gellan gum	Disintegrating agent.[36]
Tamarind seed	Binding agent, emulsifier [37]
Menthol	Penetration Enhancer [38]
Chitosan	Bioadhesive [39]
Mucilage of <i>Mimosa pudica</i>	Binding and Disintegrating agent [40]

TABLE 2: APPLICATIONS OF GUMS AND MUCILAGE'S IN NDDS

Common Name	Pharmaceutical Application
Bhara gum	Microencapsulation[41]

Cordia gum	Novel oral sustained release matrix forming agent in tablets [42]
Mucuna gum	Microspheres [43]
Sodium alginate	Bioadhesive microspheres, Nanoparticles [44]
Okra	Hydrophilic matrix for Controlled release drug Delivery[45]
Hakea Gum	Colon targeted DDS [46]
Alginates	Modulate GI Transit Time [47]

SAFETY CONCERNS : [48-50]

It is most expensive part of excipient development process. Detailed report on safety and toxicity of the excipient is needed for approval. If the excipient is in the

GRAS(generally regarded as safe) list or is from of edible source. Development of complete safety profile of excipients need some safety tests. These tests are summarized in Table 3.

TABLE 3 : SOME TESTS AND PARAMETERS FOR NEW EXCIPIENTS

Tests	Purpose
Cytotoxicity in normal as well as cancerous cell lines	Improve non toxic nature of excipient.
Histological, morphological and systemic Toxicity studies in animals.	To prove the safety of excipient.
Genotoxicity and carcinogenicity tests in animals.	To prove the safety of excipient.
Sensitization and immunogenicity tests.	To prove non- immunogenic nature of excipient.
Stress testing	To identify the degradation products within the formulations.

DEMERITS OF SYNTHETIC PHARMACEUTICAL EXCIPIENTS :

The synthetic polymers excipients have certain disadvantages such as high cost, toxicity, environmental pollution during synthesis, non-renewable sources, side effects, poor biocompatibility, release of acidic degradation products, poor processing ability and rapid loss of mechanical properties during degradation and poor

patient compliance etc. Besides these there are some more distinct evidences, like :

- Reports of adverse reactions to povidone primarily concern the formation of subcutaneous granulomas at the injection site produced by povidone. Povidone may accumulate in organs following intramuscular injections [51]

- Acute and chronic adverse effects (skin and eye irritation) have been observed in workers handling the related substances methyl methacrylate and poly- (methyl methacrylate) (PMMA) [52]
- Acute oral toxicity studies in animals have indicated that carbomer-934P has a low oral toxicity at a dose of up to 8 g/kg. Carbomer dust is irritating to the eyes too, mucous membranes and respiratory tract. For this, gloves, eye protection and dust respirator are recommended during handling[53]
- Studies and researches in rats have shown that 5% polyvinyl alcohol aqueous solution injected subcutaneously can cause anemia and can infiltrate various organs and tissues.[54]

MERITS OF NATURAL PHARMACEUTICAL EXCIPIENTS OVER THE SYNTHETIC EXCIPIENT: [55-59]

Naturally available pharmaceutical excipients mainly polymers which are used as excipients have some advantages over other available synthetic materials. Like :

- Biodegradable.
- Produced by all living organisms. They represent truly renewable source and they have no adverse impact on humans or environmental health (*e.g.*, skin and eye irritation).
- Biocompatible and non-toxic—Chemically, nearly all of these plant materials are carbohydrates composed of repeating sugar (monosaccharides) units. Hence, they are non- toxic.

- Low cost—it is always cheaper to use natural sources. The production cost is also much lower compared with that for synthetic material. India and many developing countries are dependent on agriculture.
- Environmental-friendly processing—Gums and mucilages from different sources are easily collected in different seasons in large quantities due to the simple production processes involved.
- Local availability (especially in developing countries) —In developing countries, governments promote the production of plant like guar gum and tragacanth because of the wide applications in a variety of industries.
- As most of the natural polysaccharides are degraded in the colon by intestinal micro flora there is a lot of scope for colon targeted drug delivery system. In addition natural polysaccharides are biocompatible, biodegradable, ecofriendly compared to synthetic polymers.

DEMERITS OF NATURAL GUMS AND MUCILAGES AS EXCIPIENTS : [60-62]

In spite of having some advantages over the synthetic one, the gums and mucilages used as excipients have some demerits too.

- Microbial contamination—The equilibrium moisture content present in the gums and mucilages is normally 10% or more and, structurally, they are carbohydrates and, during production, they are exposed to the external environment

and, so there is a chance of microbial contamination. However, this can be prevented by proper handling and the use of preservatives. Batch to batch variation—Synthetic manufacturing is a controlled procedure with fixed quantities of ingredients, while the production of gums and mucilages is dependent on environmental and seasonal factors.

- Uncontrolled rate of hydration—Due to differences in the collection of natural materials at different times, as well as differences in region, species, and climate conditions the percentage of chemical constituents present in a given material may vary. There is a need to develop suitable monographs on available gums and mucilages.
- Reduced viscosity on storage—Normally, when gums and mucilages come into contact with water there is an increase in the viscosity of the formulations. Due to the complex nature of gums and mucilages (monosaccharides to polysaccharides and their derivatives), it has been found that after storage there is reduced in viscosity.

CONCLUSION

Excipients are the largest components of any pharmaceutical formulation. But despite of the many potential benefits of synthetic excipients, manufacturers must still address a number of challenges before their current universe of implementation can be expanded. At the same time, research in natural polymeric materials has witnessed growing interest and attention. This is

attributable to a number of factors which include their relative abundance, low cost, biodegradable and eco-friendly profiles. Some of these natural excipients have obvious advantages over their synthetic counterparts in some specific delivery systems due to their inherent characteristics.

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Evaluation of Antiulcer Activity of Ethanolic Fruits Extract of *Ficus Carica*

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Abstract

The present study was undertaken to ascertain the antiulcer activity of *Ficus carica* fruit extract in pylorus ligation model using albino wistar rats. The ethanolic extract of *Ficus carica* fruit was given in a dose of 100 and 200 mg/kg body weight in rats. Total acidity, free acidity, pH, ulcer index and % ulcer inhibition were measured in this model. Our present study revealed that the ethanolic fruit extract had good antiulcer activity.

Key words: Antiulcer, *Ficus carica*, β -sitosterol.

Introduction

Ulcer is one of the most common and important problem among the peoples in developing countries. Recently, commercially available synthetic drugs are very costly and produced higher side effects as compare to drugs obtained from natural resources, as they are cheap, easily available and less toxic. About 70% population of developing countries relies on traditional medicine for their primary health care [1].

Ficus carica is important world crop belongs to the family Moraceae. The synonyms of *Ficus carica* is Fig in English, Anjeer in Arabic and Urdu. It has been cultivated in various places worldwide as an edible fruit. Various parts of plant like bark, leaves, fruit, seeds, tender, root and latex are also important for medicinal properties. The dried fruits are rich in calcium, fiber, magnesium, copper, manganese, vitamin-K and potassium. They are good source of flavonoids, polyphenols and some bioactive compounds such as arabinos, β -amyris, β -sitosterol, β -carotenes, glycosides and xanthotoxol. Traditionally *Ficus carica* root is used in the treatment of leucoderma and ringworm infections and fruit has antipyretic, purgative, aphrodisiac properties and are used in the treatment of inflammation and paralysis [2]. *Ficus carica*

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has been reported for its antiulcer, antioxidant, antiviral, antibacterial, hypoglycemic, anticancer, hypotriglyceridaemic, and anthelmintic properties. β -sitosterol is a naturally occurring terpenoid which has ulcer protective activity. Since, all parts of *Ficus carica* plant contain β -sitosterol which may have ulcer protective activity [2]. This observation allowed us performing ulcer protective action of ethanolic fruit extract of *Ficus carica*.

Materials and method

Plant material

The fruits of *Ficus carica* used in this study were purchased from local market at Lucknow, Uttar Pradesh. The dried fruits were authenticated by Department of Applied Plant Science (Horticulture), Babashahib Bhimrao Ambedkar University, Lucknow, India and voucher sample was deposited in the Department. Fruits were air dried, ground to powder and stored in an air tight container.

Preparation of plant extract

Accurately 500 gm of powdered sample of *Ficus carica* fruit was extracted in Soxhlet apparatus using ethanol as a solvent. The ethanolic extract was concentrated under reduced pressure using a rotary evaporator until complete drying. The dried powder of the extract was stored at temperature 4°C. The percentage yield of extract was found to be 3.7%.

Animals

Albino rats weighing (100-150g) were procured from BBDNIIT, Lucknow. The animals were kept in polypropylene cages under standard condition of temperature at $25 \pm 1^\circ\text{C}$ with 12 h light/dark cycle and had a free access to commercial pellet diet and

water. The animals were given one week time to become acclimatized with laboratory condition before the experiment. The study was approved by the Institutional Animal Ethics Committee (Reference No.: BBDNIIT/ IAEC/16/2012).

Drugs and chemicals

Pantoprazole was kindly donated by Alembic Pharmaceuticals, Vadodara, Gujrat, India. All other chemicals of analytical grade were purchased from Himedia Chemicals, India.

Method (Pyrolus ligation)

Albino wistar rats of either sex (100-150g) were divided into four groups having six animals in each. Group I served as control and received normal saline (3 ml/kg, po); group II served as pyrolus ligation control (toxic control) taking normal saline (3 ml/kg, po); group III served as standard and received pantoprazole (10mg/kg, po); group IV and V received *Ficus carica* ethanolic extract (100mg/kg and 200mg/kg, po, for seven days), respectively. After seven days, ligation of the stomach at pyrolic end was performed using ether anesthesia. Animals were sacrificed approximately 4 h later and stomach was opened to collect gastric content. The volume of gastric juice was measured and centrifuged at 2000 rpm for 10 min to separate the supernatant. Aliquots (1 ml of each) were taken for the determination of pH, total acidity and free acidity. The inner surface of isolated stomach was examined for gastric lesions [3].

Biochemical estimations [4]

Determination of pH

An aliquot of 1ml gastric juice was diluted with 1ml of distilled water and pH of the solution was measured using a pH meter.

Determination of Total acidity

An aliquot of 1ml gastric juice was diluted with 1ml of distilled water and was taken into a 50ml conical flask. Two drops of phenolphthalein indicator was added to it and titrated with 0.01(N) sodium hydroxide. The total acidity was expressed as meq/l by the following formula:

$$\text{Total acidity} = n \times 0.01 \times 39.99 \times 1000$$

Where n is volume of sodium hydroxide consumed, 39.99 is molecular weight of sodium hydroxide, 0.01 is normality of sodium hydroxide, and 1000 is the factor.

Determination of Free acidity

$$\text{Free acidity} = n \times 0.01 \times 39.99 \times 1000$$

Where n is volume of sodium hydroxide consumed, 39.99 is molecular weight of sodium hydroxide, 0.01 is normality of sodium hydroxide, and 1000 is the factor [5].

Statistical analysis

All the data are presented as mean \pm SD and analyzed by one way ANOVA using Bonferroni multiple comparison test. $P < 0.05$ was considered statistically significant. Statistical analysis was carried out using Graphpad Prism 3.0 (Graph pad software, San Diago, CA).

Results and discussion

The ethanolic extract of *Ficus carica* in dose of 100 and 200 mg/kg produced significant reduction in ulcer index, gastric volume, free acid, total acid and increase in pH in comparison with control group (Table 1). The present investigation revealed the anti ulcer activity of ethanolic extract of *Ficus carica*. It was evaluated against pylorus ligation induced gastric ulceration. These models represent the most common causes of ulcer in human. The ethanolic extract of *Ficus carica* showed decreased in both free and total acidity, gastric volume content in pylorus ligation model which is comparable with standard pantoprazole. It was also observed that there was a decrease in ulcer index and % inhibition of ulcer. It is obvious that the extract of two doses (100 and 200 mg/kg) have significant effects on ulcer healing properties. It may be postulated that β -sitosterol was extracted from fruits using ethanol which showed dramatic effect on ulcer protection. Therefore, this ethanolic extract of *Ficus carica* fruits may be used for ulcer inhibition for future. Further studies like various biochemical estimations and histopathological studies should be necessary to confirm the ulcer protective activity.

Table 1. Effect of ethanolic fruit extract of *Ficus carica* on gastric content, pH, free acidity, total acidity, ulcer score, ulcer index, and % ulcer inhibition in pylorus ligation induced ulcer.

Group	Treatment	Gastric Content(ml)	pH of gastric content	Free acidity (mEq/l)	Total acidity (mEq/l)	Ulcer index	% Ulcer inhibition
1	Control	2.39 ± 0.07	3.17 ± 0.05		25.86 ± 1.22	5.46 ± 0.94	-
2	Toxic control	3.08 ± 0.078	2.00 ± 0.09		31.28 ± 1.37	36.24 ± 1.76	-
3	Pantoprazole (10 mg/kg BW)	1.51 ± 0.067	3.27 ± 0.09		27.14 ± 0.55	12.49 ± 0.87	65.53 ± 1.62
4	<i>Ficus carica</i> (100 mg/kg BW)	1.94 ± 0.058	2.87 ± 0.12 ^a		28.38 ± 0.78 ^a	15.78 ± 1.51	56.46 ± 1.21
5	<i>Ficus carica</i> (200 mg/kg BW)	2.09 ± 0.08 ^a	2.99 ± 0.09 ^b		26.58 ± 0.51	13.85 ± 1.14	64.55 ± 1.59

Data was represented as Mean ± SD. In the following Table 1, comparisons were made on the basis of one-way ANOVA and followed by Bonferroni multiple comparison method and data was statistically significant when $a < 0.01$ and $b < 0.05$ with respect to control groups (n=6).

Conclusion

Gastric ulceration is the common and important problem worldwide. However, different kinds of synthetic drugs are available, but uses of traditional system of medicine also become important because of their less toxicity profile. In the present study, ethanolic fruit extract of *Ficus carica*

exhibits potential antiulcer activity in pylorus ligation induced gastric ulceration over the standard synthetic drug pantoprazole.

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Determination of Parent ion & Daughter ion of Cefuroxime by ESI-MS-MS

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Abstract

Now a day's mass spectrometer has become a very important part in the field of drug discovery. In this paper a reconstructed ion chromatogram is being generated by mass spectrometer. AB SCIEX API 2000 MS-MS system by electro-spray ionization (ESI) mass spectrometry with multiple reaction monitoring (MRM) of positive ion mode has generated a parent ion 423.1 and daughter ion 206.8.

Key words: Tandem mass spectrometry (MS/MS), ESI, Ionization

Introduction

Mass spectrometry using ESI is called electro spray ionization mass spectrometry (ESI-MS) or, less commonly, electro spray mass spectrometry (ES-MS). ESI is a so-called 'soft ionization' technique. Tandem mass spectrometry (MS/MS) is a combination of two MS. The aim is to get the structural information by fragmentation of the ions isolated or to get better selectivity and sensitivity for quantitative analysis by selecting representative ion transitions [1]. This work has shown the base peak of a certain drug. The base peak is almost similar to the molecular weight of the drug. In this study cefuroxime has been taken to show the molecular weight by using MS. To specify the compound identity, daughter ion is being generated by MS-MS.

Experimentation

Literature review has provided the physiochemical property of cefuroxime [2]. Then the drug is being dissolved into methanol. 100 ng/ml concentration of cefuroxime is prepared by serial dilution. Then Hamilton syringe is used to inject the drug into MS-MS. In MS-MS the syringe pump mode is being activated. Then all the parameters are optimized. AB SCIEX API 2000 MS-MS system by electro spray ionization (ESI) mass spectrometry with multiple reaction monitoring (MRM) of positive ion mode has generated a parent ion 423.1 and daughter ion 206.8. Analyst 1.4.2 software was used to generate the data.

Skimmer cone: a cone with a sampling orifice of reduced diameter to preferentially sample gas phase ions and reduce the gas load entering the vacuum system of the mass analyzer device.

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Mass analyzer-this deflects ions down curved tubes in a magnetic fields based on their kinetic energy determined by the mass, charge and velocity. The magnetic field is scanned to measure different ions.

Quadrupole-this device that uses electric fields in order to separate ions according to their mass to charge ratio (m/z) as they pass along the central axis of four parallel equidistant rods. A quadrupole mass analyzer consists of four parallel rods arranged in a square. The analyte ions are directed down the center of the square. Voltages applied to the rods generate electromagnetic fields. These fields determine which mass-to-charge ratio of ions can pass through the filter at a given time. Quadrupoles tend to be the simplest and least expensive mass analyzers [3,4].

Quadrupole mass analyzers can operate in two modes:

- ❖ Scanning (scan) mode
- ❖ Selected ion monitoring (SIM) mode

Collision cell-ions emerging from the first mass analyser are accelerated using a potential difference and collide with neutral gas molecules such as H_2 , N_2 etc causing analyte fragmentation.

Detection-once produced and separate, the ions need to be detected and transformed into a usable signal. Electron multiplier is used for detection. Amplified current measured and related to ion count and it allows a sensitive and rapid scanning

Vacuum system-mass analysers require high levels of vacuum in order to operate in a predictable and efficient way.

Mass spectrometers work by ionizing molecules and then sorting and identifying the ions according to their mass-to-charge (m/z) ratios [5,6].

Result and Discussion

Data Acquisition Modes

- Simple MS Scanning---Using one quadrupole
- Product ion scan (daughter)--MS1 static MS2 scanning
- Precursor ion scan (parent)--MS1 scanning MS2 static
- Neutral loss scan--MS1 and MS2 scanning but synchronised
- Multiple reaction monitoring (MRM)--MS1 and MS2 static and enhanced sensitivity

Source Optimization Parameters

- Ion Source- Electrospray ionization
- Polarity-positive mode
- Gas 1 - Helps generate small droplets of sample flow
- Gas 2 - Turbo gas, helps evaporate the spray droplets and prevents solvent entering the system
- Temperature - Temperature of the turbo gas was 300°C
- Gas flow – 5 l/min
- Curtain Gas - Prevents solvent droplets from entering and contaminating the ion optics
- Ion Spray voltage - The voltage applied to the needle that ionizes the sample at the ion source was 3000v
- Nebulizer or needle current - The current applied to the corona discharge needle was 1000v
- Interface Heater - prevents contamination of ion optics

Compound Dependent Parameters

- De-clustering potential (DP) - the potential difference between the ground (usually the skimmer) and the orifice plate. Used to minimize solvent cluster ions, which may attach to the sample. The higher the voltage the greater the amount of fragmentation

- Entrance Potential (EP)- Focuses the ions through the high pressure Q0 region
- Collision cell entrance potential (CEP)- Focuses ions into the collision cell
- Collision Energy (CE) - The amount of energy precursor ions receive as they are accelerated into the collision cell.
- Collision Gas (CAD)
- Collision Cell Exit Potential (CXP)

All the parameters are optimized to reproduce the ionization method. As the parameters are acquired in that particular value and that method was being developed to generate the base peak from the total ion count. By optimizing the source parameters, ionization takes place and the parent ion is found at 423.1 with 6.7e4 intensity [Fig 1]. The ions observed by mass spectrometry may be quasimolecular ions created by the addition of a hydrogen cation and denoted $[M + H]^+$, or of another cation such as sodium ion, $[M + Na]^+$, or the removal of a hydrogen nucleus, $[M - H]^+$. Multiply charged ions such as $[M + nH]^{n+}$ are often observed. This method was developed in positive mode so addition of a hydrogen cation can take place. This report shows the molecular weight of the molecule. By this

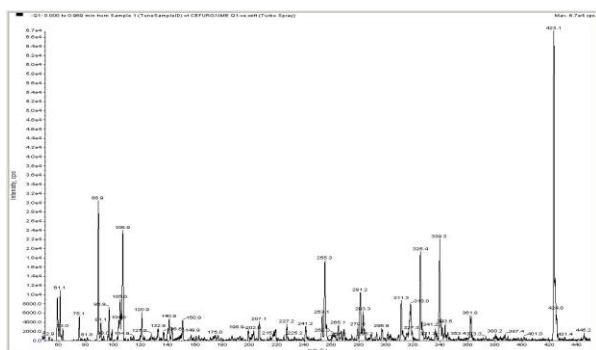
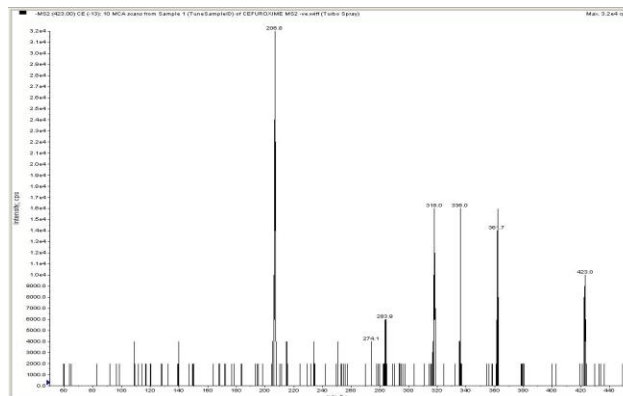


Fig1: Parent ion of cefuroxime 423.1 by Q1 scan

method we can understand the conventional process to determine the molecular weight

of the certain molecule by Mass Spectrometry. Analyst 1.4.2 software was used to generate the data.

Compound based parameters are optimized



for fragmentation. Once the base peak of the parent ion (Q1) was generated then the molecule entered in to Q2 cell where the collision takes place. All the compound based parameters were optimized to isolate the fragile part of the structure which gives the most stable and reproducible peak is chosen as the daughter ion. As all the parameters are optimized, fragmentation occurs always with the same part.

Fig 2: Daughter ion of cefuroxime 206.8 by Q3 scans

Cefuroxime was taken as a model drug to focus on the method to determine the parent and daughter ion pair. Parent ion of cefuroxime 423.1 by Q1 scan → Daughter ion of cefuroxime 206.8 by Q3 scan. This ESI-MS-MS method gets advantage over all the analytical technique because of its selectivity and specificity.

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Medicinal application of different parts of *Nyctanthes arbortristis*

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Abstract: With the growth of population cost of treatment, inadequate supply of drugs, side effect of several allopathic drugs and development of resistant to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Some forest species are used as raw materials for manufacture of drugs and perfumery products due to medicinal properties. The present investigation *Nyctanthes arbortristis*, commonly known as Harsinghar, Shiuli, Night Jasmine, Coral Jasmine etc., belongs to the oleaceae family. *Nyctanthes arbortristis* is widely distributed shrub. The leaves of *Nyctanthes arbortristis* are antibacterial, anti-inflammatory and anthelmintic. The flowers are bitter astringent, ophthalmic, stomachic and carminative. It is expectorant, bitter and tonic, febrifuge and mild purgative. It is used as bilious and obstinate remittent fever, sciatica and rheumatism. It is used in the treatment of dry cough, fungal skin infection, bronchitis.

Key-words: *Nyctanthes arbortristis*, medicinal plants, drugs, Harsinghar, Shiuli

Introduction: Nearly all cultures on the earth use medicinal plants and herbs for the prevention or treatment of their illness. As per WHO estimation, nearly 80% of the population in the many

Asian and African countries depends on herbal medicine. The same percentage has used in the form of traditional or medicinal therapy, in developed countries. *Nyctanthes arbortristis* is commonly known as Harsinghar, Harsingar, Harashringara in Hindi, Shefali, Shephalika, Siuli in Bengali, Sewali in Assamese, Prajakta, Prajakt, Parijatha in Sanskrit. The tree is sometimes called the tree of sorrow, because the flowers lose their brightness during day time. The name arbor-tristis means sad tree. Its flower is the official flower of the state of West Bengal, India, and for Kanchanaburi Province, Thailand [1-5].

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Methods: A survey of medicinal plants used by the rural and urban inhabitants of Banka district, state of Bihar was performed by means of 100 interviews with medicinal plant users.

Results and discussion:

Medicinal use of *Nyctanthes arborescens* (Harsinghar):

Leaves:

In Ayurvedic medicine leaves of *Nyctanthes arborescens* are used for the treatment of different diseases such as eumatism, internal worm infections, chronic fever. Leaf juice with honey is given internally thrice daily for the treatment of dry cough. The leaf juice is also used for the treatment of chronic fever, rheumatism, sciatica, liver disorders. The leaf juice with common salt is used for the treatment of intestinal worms. The aqueous paste of leaves is used externally for the treatment of skin related troubles, especially for the treatment of ring-worm. Paste of Leaves with honey reported for the treatment of high blood pressure and diabetes [6], enlargement of spleen [7, 8].

Seeds:

Seeds are used as anthelmintics. It is antibilious and an expectorant and is used for the treatment of bilious fevers. The seeds are used to cure scurfy affections of scalp, piles and skin diseases. The seeds are crushed and aqueous paste is prepared. The patients are suffering from piles are advised to apply fresh paste externally on piles, along with the internal use of the powdered seeds. This treatment is simple and very effective. The decoction of seeds is used as

hair tonic and advised to wash the hair daily in order to get rid from dandruff and lice.

Flowers:

Flowers are used as antibilious, expectorant, carminative, stomachic, hair tonic and for the treatment of piles, skin diseases and ophthalmic purposes. The decoction of flowers is used for the treatment of gout. The decoction is given up to one week during the time of attack. As treatment, it is given up to one month in a year. Flowers are sweet scented, sessile, 3-7 together in pedunculate heads which are arranged in short trichotomous cymes; bracts elliptical. The bright orange corolla tubes of the flowers contain a colouring substance were formerly used for dyeing silk.

Stem:

The powder of stem bark is used for the treatment of rheumatic joint pain, bronchitis and malaria. Reported that the bark is used for the treatment of snakebite and bronchitis [9, 10]. The paste of stem bark of *Nyctanthes arborescens* along with Arjuna bark is rubbed on the body for the treatment of joint broken bones [11].

Description:

Nyctanthes arborescens (Harsinghar): *Nyctanthes arborescens* is a deciduous tree with quadrangular branches and grey or greenish-white rough bark. Leaves opposite, 10-13 by 6-8 cm ovate, acute, coriaceous, covered with stiff white hair; base rounded or cuneate. Flowers are sweet scented, sessile, 3-7 together in pedunculate heads which are arranged in short trichotomous

cymes; bracts elliptical. Calyx-tube 5 cm, minutely 4-5 toothed. Corolla-tube cylindrical, orange-red; limb white, spreading; emarginated, contorted in bud. Anthers 2, subsessile, inserted near the mouth of the corolla tube. Ovary 2-celled; ovule 1 in each cell, erect. Capsule long, orbicular, compressed, chartaceous, 2-celled; seedsexualbuminous.

Conclusion:

Nyctanthes arbortristis is widely distributed shrub useful for the treatment of dry cough, fungal skin infection, bronchitis, sciatica and rheumatism. Leaves are antibacterial, anti-inflammatory and anthelmintic. The flowers are bitter astringent, ophthalmic, stomachic and carminative. It is expectorant, bitter and tonic, febrifuge and mild purgative.

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