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Identification of Secondary Metabolites Present in Carica Papaya L. Leaf Extract Found in Northern India by HPTLC & Other Test Procedures, its Significance and Role in Curing Dengue/DHF Disease

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ABSTRACT

The present study was performed for the development of physico-chemical parameters of Carica papaya Linn. Leaves (CP) belong to family Caricaceae. The study comprises physico-chemical and phytochemical evaluation to confirm the Secondary metabolites present in Carica papaya leaf. The TLC fingerprinting profile of different extracts developed which exhibited the presence of several secondary metabolites. The methods adopted here for the identification of Secondary Metabolites is simple, specific, accurate and rapid also. Extraction from the plant is an empirical exercise in which different solvents are utilized under a variety of conditions such as time and temperature during extraction. After extraction, the bioactive components were separated from the co extractives. Further steps for the isolation involved is the solvent partition of the co extractives of chromatographic methods in order to fractionate the compounds based on their acidity, polarity or molecular size and Qualitative Methods also. For purification, some structural analysis, may be accomplished by appropriate techniques such as recrystallisation, sublimation, distillation/extraction. Here, the distillation process was done. The main purpose for this study is to find out the role of phytochemicals, particularly secondary metabolites present in Carica papaya L. leaves in curing the disease Dengue/DHF directly or indirectly.

KEYWORDS : *Carica papaya* L., CP, TLC, HPTLC, Primary Metabolites & Secondary Metabolites

Introduction

The molecules, e.g., lipids, proteins, nucleic acids. and carbohydrates are called *Primary Metabolites*. Unlike animals, however, most plants divert a significant proportion of assimilated carbon and energy to the synthesis of organic molecules that may have no obvious role in normal cell function. These molecules are known as *Secondary Metabolites*. Metabolites are organic compounds synthesised by organisms using enzyme-mediated chemical reactions called metabolic pathways. The secondary metabolites are variously distributed in the plant kingdom, and their functions are specific to the plants in which they are found. Secondary metabolites are often coloured, fragrant or flavourful compounds; they typically mediate the interaction of plants with other organisms. Such interactions include those of plant-pollinator, plant-pathogen, and plant herbivore. Some primary metabolites are precursors (starting materials) for the synthesis of secondary metabolites. they which are largely fall into three classes of compounds; alkaloids, terpenoids, and phenolics. However, these classes of compounds also include primary metabolites, so whether a compound is a primary or secondary metabolite is a distinction based not only on its chemical structure but also on its function and distribution within the plant kingdom because many thousands of secondary metabolites have been isolated from plants, and many of them have powerful physiological effects in humans and are widely used as medicines. It is only since the late twentieth century that secondary metabolites have been clearly recognized as having important functions in plants. Research has focussed on the role of secondary metabolites and the main purpose for this study is to find out the role of phytochemicals, particularly secondary metabolites present in *Carica papaya* L. leaves in curing the disease like Dengue directly or indirectly.

Tannins

A tannin (also known as vegetable tannin, natural organic tannin or sometimes tannoid, i.e. a type of biomolecule, as opposed to modern synthetic tannin) is an astringent, bitter plant polyphenolic compound that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids. The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation and perhaps also as pesticides and in plant growth regulation.[1] The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripened fruit or red wine.[2] Likewise, the destruction or modification of tannins with time plays an important role in the ripening of fruit and the ageing of wine.

Saponins

Saponins are a class of chemical compounds, one of many *secondary metabolites* found in natural sources, with saponins found in particular abundance in various plant species. More specifically, they are *amphipathic glycosides* grouped, in terms of *phenomenology*, by the soap-like foaming they produce when shaken in *aqueous* solutions, and, in terms of structure, by their composition of one or more *hydrophilic* glycoside moieties combined with a *lipophilic triterpene* derivative.[3][4] The Commercial formulations of plant-derived saponins, e.g., from the soap bark (or soapbark) tree, *Quillaja saponaria*, and those from other sources are available *via* controlled manufacturing processes, which make them of use as chemical and biomedical reagents.

Phlobatannins

Phlobatannins, a tannin with hot dilute acids yields a *phlobaphene* origin of *Phlobatannins phloba*phene + *tannin.* Phlobaphenes are reddish, alcohol soluble and water insoluble phenolic substances. They can be extracted from plants or products that result from treatment of tannin extracts with *mineral acids* (tanner's red).[5]

Flavonoids

The scientific evidences for the hypothesis that dietary flavonoids promote health and prevent disease in humans. Flavonoids connected to one or more sugar molecules are known as flavonoid *glycosides*, while those that are not connected to a sugar molecule are called aglycones. With the exception of flavanols (catechins and proanthocyanidins), flavonoid occur in plants and most foods as glycosides. [6] Even after cooking, most of the flavonoids glycosides reach the small intestine intact. Only flavonoid aglycones and flavonoid glucosides (bound to glucose) are absorbed in the small intestine, where they are rapidly metabolized to form methylated, glucuronidated, or sulphated *metabolites*.[7] Bacteria that normally colonise the *colon* also play an important role in flavonoid metabolism and absorption. Flavonoids or flavonoid metabolites that reach the colon may be further metabolized by bacterial *enzymes*, and then absorbed. A person's ability to produce specific flavonoid metabolites may vary and depends on the milieu of the colonic microflora. [8] [9] In general, the *bioavailability* of flavonoids is relatively low due to limited absorption and rapid elimination. Bioavailability differs for the various flavonoids. isoflavones are the most bio available group of flavonoids, while flavanols (proanthocyanidines and tea catechins) and anthocyanins are very poorly absorbed. [10] Since flavonoids are rapidly and extensively metabolized, the biological activities of flavonoid metabolites are not always the same as those of the parent compound. [11] When evaluating the data from flavonoid research in cultured cells, it is important to consider whether the flavonoid concentrations and metabolites used are physiologically relevant. [12] In humans, peak *plasma* concentrations of soya isoflavones and citrus flavanones have not been found to exceed 10 micromoles/liter after oral consumption. Peak plasma concentrations measured after the consumption of anthocyanins, flavanols and flavonols (including those

from tea) are generally less than 1 micromole/liter. Flavonoids are effective scavengers of free radicals in the test tube (*in vitro*). [13][14] However, even with very high flavonoid intakes, *plasma* and intracellular flavonoid concentrations in humans are likely to be 100-1,000 times lower than concentrations of other antioxidants, such as ascorbate (vitamin C), uric acid, or glutathione. Moreover, most circulating flavonoids are actually flavonoid metabolites, some of which have lower antioxidant activity than the parent flavonoid. For these reasons, the relative contribution of dietary flavonoids to plasma and tissue antioxidant function in vivo is likely to be very small or negligible.[11][15][16] Decreasing platelet aggregation, [17][18] which is one of the first steps in the formation of a blood clot can occlude a coronary or cerebral artery, resulting in myocardial infarction or stroke, respectively. Inhibiting platelet aggregation is considered an important strategy in the primary and secondary prevention of cardiovascular disease.[19] Vascular endothelial cells play an important role in maintaining cardiovascular health by producing nitric oxide, a compound that promotes arterial relaxation (vasodilation). [20] Arterial vasodilation resulting from endothelial production of nitric oxide is termed endothelium-dependent vasodilation. Several clinical trials have examined the effect of flavonoid rich foods and beverages on endothelium dependent vasodilation. Endothelial nitric oxide production also inhibits the adhesion and aggregation of platelets, one of the first steps in blood clot formation.[20] A number of clinical trials have examined the potential for high flavonoid intake to decrease various measures of platelet aggregation outside the body (ex vivo).

Anthraquinones

The anthraquinones often occur in plants in the form of glycosides. Emodin is the most widely occurring anthraquinone in medicinal herbs. Among Chinese herbs, it is found in several species of *Rheum*, *Rumex*, *Polygonum*, and *Cassia*. Valuable medicinal aspects of emodin have been proclaimed on the basis of laboratory research, including: potential cancer prevention activity (blocking the growth of newly transformed cells); antiviral activity; antioxidant properties; gastric ulcer protection; liver protection; promoting blood circulation; inhibiting autoimmune attack; and benefiting the kidneys (inhibiting undesired proliferation of cells). The anthraquinones and their metabolites are retained in the macrophages, where they yield the dark colour. Melanosis coli could also arise more rarely from serious diseases of the intestine, the blackening coming from areas of cell necrosis. Thus, the laxative-related condition is sometimes called pseudomelanosis coli, to indicate that the darkening of the intestinal wall is believed only due to to discoloration rather than a physiological disorder. A debate over the significance of melanosis coli in relation to anthraquinone use soon arose, and it persists to till today. On the one side, many doctors have interpreted this condition as one of the simple staining of the intestinal wall by anthraquinone residues, and viewed it as a harmless and reversible condition. [21][22][23][24]

Terpenoids

Terpenoids (also called isoprenoid) constitute one of the largest families of natural products accounting for more than 40,000 individual compounds of both primary and secondary metabolisms. Most of them are of plant origin, and hundreds of new structures are reported every year.[25][26] Particularly, terpenoids are contained in many herbal plants, and several terpenoids have been shown to be available for pharmaceutical applications, for example, artemisinin and taxol as malaria and cancer medicines, respectively. Various terpenoids are contained in many plants not only for herbal medicine use but also for dietary use. [27]

Steroids

The steroids vary by the functional groups attached to its four-ring core and by the *oxidation state* of the rings. *Sterols* are special forms of steroids, with a *hydroxyl* group at position-3 and a skeleton derived from *cholestane*. [28] Hundreds of distinct steroids are found in *plants*, *animals* and *fungi*. All steroids are made in cells either from the sterols *lanosterol* (animals and fungi) or from *cycloartenol* (plants). Both lanosterol and cycloartenol are derived from the cyclization of the *triterpene squalene*. [29] Steroids are a class of *organic compounds* with a chemical structure that contains the core of *gonane* or a skeleton derived there from. Usually, *methyl groups* are present at the carbons C-10 and C-13 – an *alkyl side-chain* at carbon C-17 may also be present. *Gonane* is the simplest possible steroid and is composed of seventeen *carbon* atoms bonded together to form four fused rings.

Glycosides

In chemistry, a glycoside is a molecule in which a sugar is bound to another functional group via a glycosidic bond. Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis,[30] which causes the sugar part to be broken off, making the chemical available for use. Many such plant glycosides are used as medications. In animals and humans, poisons are often bound to sugar molecules as part of their elimination from the body. In formal terms, a glycoside is any molecule in which a sugar group is bonded through its anomeric carbon to another group via a glycosidic bond. Many authors require in addition that the sugar be bonded to a non-sugar for the molecule to qualify as a glycoside, thus excluding polysaccharides. The sugar group is then known as the glycone and the non-sugar group as the aglycone or genin part of the glycoside. The glycone can consist of a single sugar group (monosaccharide) or several sugar groups (oligosaccharide). The first glycoside ever identified was amygdalin, by the French chemists Pierre Robiquet and Antoine Boutron-Charlard, in 1830.[31] Depending on whether the glycosidic bond lies "below" or "above" the plane of the cyclic sugar molecule, glycosides are classified as α-glycosides or β-glycosides.

Alkaloids

The primary metabolites from which they are derived include amino acids such as tryptophan, tyrosine, and lysine. Alkaloid biosynthetic pathways can be long, and many alkaloids have correspondingly complex chemical structures. Alkaloids accumulate in plant organs such as leaves or fruits and are ingested by animals that consume those plant parts. The term 'bioavailability enhancer was first coined by Indian scientists at the Regional Research Laboratory, Jammu (RRL), now known as Indian Institute of Integrated Medicine, Jammu), who discovered and scientifically validated piperine as the world's first bioavailability enhancer in 1979.[32]

Mechanism of Bio-enhancer, Piperine

Different mechanisms as the bio-enhancer activity of piperine have been proposed including DNA receptor binding, modulation of cell signal transduction and inhibition of drug efflux pump.[33]In general, it inhibits drug metabolising enzymes, stimulates absorption by stimulating gut amino acid transporters, inhibits the cell pump responsible for drug elimination from cells and inhibits intestinal production of glucuronic acid. It may increase the absorption of drug in the GIT, or inhibit enzymes responsible for drug metabolism, especially in the liver when the drug passes through the liver after absorption from GIT. Oral administration of piperine in rats strongly inhibited the hepatic arylhydrocarbon hydroxylase and UDP-glucuronyltransferase activities.[34] Another study demonstrates that piperine modifies the rate of glucuronidation by lowering the endogenous UDP-glucuronic acid content and also by inhibiting the transferase activity. [35] Piperine inhibits human P-glycoprotein and cytochrome P450 3A4 (CYP3A4).[36]Both the proteins contribute to a major extent to first-pass elimination of many drugs. Some of the metabolising enzymes inhibited or induced by piperine include CYP1A1, CYP1B1, CYP1B2, CYP2E1, CYP3A4 etc. Most of the drugs metabolised by these enzymes will therefore be influenced by bio-enhancers. Some other suggested mechanisms include making target receptors more responsive to drugs, acting as receptors for drug molecules, increasing GIT vasculature by vasodilation to increase absorption of drugs, modulation of the cell membrane dynamics to increase transport of drugs across cell membranes. [37]

Materials and Methods

Aqueous extract, organic extract, water bath, distilled water, Whatman Filter paper No1, 1% lead acetate, 10% ammonia solution, chloroform, acetic acid, Mayer's and Wagner's reagent, Molisch's reagent, iodine solution etc.

All chemicals/reagents were purchased from E. Merck Ltd., Mumbai, India which were used for the successive digestion/extraction during the experiment.

Collection and preparation of Plant material

Six samples of fresh leaves without petioles were collected from plain area of Northern India, National Capital Region (NCR) and Shimla, Himachal Pradesh, from hills, height about 2205 m or 7234 ft from the sea level was chosen for the collection in the month of July, 2011(rainy season in India). The plant material was authenticated and identified

from Raw Materials Herbarium and Museum at National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India. All the samples were washed thoroughly with distilled water and dried in air under shade at room temperature for 30-45 days. Completely dried samples were grounded into fine powder mechanically using an electric blender then sieved by using a very fine muslin cloth. Finely powdered samples were then stored in air tight containers at ambient temperature until required.

Phytochemical analysis

Phytochemicals were determined by aqueous extract in deionised water and in ethanol for organic extract, after the complete extraction/ digestion, filtered by ashless Whatman Filter paper No.1 with the pore size 20-25 µm by using the most advanced and sophisticated instrument i.e. High Performance Thin Layer Chromatography (HPTLC) with Photo documentation.

Solvent Extraction Method

20 g of the powdered leaves were weighed, tied up in filter papers and put into the thimble. A reflux condenser and a round bottom flask were fitted above and below the thimble respectively. This assembly of apparatus, known as a Soxhlet extractor, was clamped firm into position and 250 ml of absolute ethanol poured into the round bottom flask. The Soxhlet extractor was then heated electrically on a heating mantle. A continuous extraction was carried out for a period of 8 h with about eighteen refluxes. The samples were then removed and the ethanol recovered. The sample(s) were poured into bijou bottles and the flasks washed with some quantity of ethanol and transferred into the bottles. These were later evaporated on the heating mantle and stored in a refrigerator.

Aqueous Extraction Method; (By Steam distillation)

20 g of the powdered leaves were placed in round bottom flasks and 200 ml of water added to each. The resulting suspensions were heated on a heating mantle. The steam-volatile oils volatilized with the steam and were condensed and collected in conical flasks as distillates. The distillation process was carried out for a period of 2 h. The oils settled on top of water were removed with the aid of separating funnel. The lower layer contains phytochemicals which are soluble in water.

General Tests; Qualitative Tests:
Liebermann's test: for glycone
Salkowski's test: for steroidal nucleus; Glycone
Molisch Test: for Carbohydrates
Fehling's Test: for reducing Sugars
Keller-kiliani test: for cardenolides

Results

(The results of phytochemical analysis by qualitative test(s) of Carica papaya L. leaves of 6 samples given in the following Table-1)

Table-1Phytochemical Screening of *C. papaya* L. Leaves by Qualitative Test

Constituents	GLP	GLH	BLP	BLH	YLP	YLH
Tannins (Aq.)	Present	Present	Present	Present	Present	Present
Saponins (Aq.)	Present	Present	Present	Present	Present	Present
Phlobatannins (Aq.)	Present	Present	Present	Present	Present	Present
Flavonoids (Aq.)	Present	Present	Present	Present	Present	Present
Anthraquinones (Aq.)	Absent	Absent	Absent	Absent	Absent	Absent
Terpenoids (Org.)	Present	Present	Present	Present	Present	Present
Steroids (Aq.)	Absent	Absent	Present	Absent	Present	Present
Alkaloids (Aq.)	Present	Present	Present	Present	Present	Present
For Carbohydrates;						
Molischs Test	Present	Present	Present	Present	Present	Present
Iodine	Absent	Absent	Absent	Absent	Absent	Absent
For Glycosides;						
Liebermanns Test (Org.)	Absent	Absent	Present	Absent	Present	Present
Salkowskis Test (Aq.)	Present	Present	Present	Present	Present	Present
Killer Killiani (Aq.)	Present	Present	Present	Present	Present	Present

(Abbreviations: GLP-Green Leaves from Plains, GLH-Green Leaves from Hills, BLP- Brown Leaves from Hills, BLH- Brown Leaves from Plains, YLP- Yellow Leaves from Plains, YLH- Yellow Leaves from Hills, Org,- Organic Solvent, Aq.- Aqueous Solvent).

Identification of Phytochemicals (Secondary Metabolites) which were analysed by the HPTLC Technique

Thin-layer chromatography (TLC) or High Performance Thin Layer Chromatography (HPTLC) is particularly valuable for the qualitative as well as quantitative determination of small amounts of impurities. As this method is effective and easy to perform, and the running cost of the equipment is cost effective, the technique is frequently used for evaluating herbal materials and their preparations. The Chromatographic fingerprint of an Herbal Medicine (HM) is a chromatographic pattern of the extract of some common chemical components of pharmacologically active and or chemical characteristics. This chromatographic profile should be featured by the fundamental attributions of integrity and fuzziness or similar or different so as to chemically represent the HM investigated. It is suggested that with the help of chromatographic fingerprints obtained, the authentication and identification of herbal medicines can be accurately conducted (integrity) even if the amount and/or concentration of the chemically characteristic constituents are not exactly the same for different samples of this HM (hence, fuzzines) or, the chromatographic fingerprints could demonstrate both the similar and different between various samples successfully. Thus, we should globally consider multiple constituents in the HM extracts, and not individually consider only one and/or two marker components for evaluating the quality of the HM products. However, in any HM and its extract, there are hundreds of unknown components and many of them are in low amount. Moreover, there usually exists variability within the same herbal materials. Hence it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic components of the HM. In the phytochemical evaluation of herbal drugs, TLC is being employed extensively for the following reasons: (1) it enables rapid analysis of herbal extracts with minimum sample clean-up requirement, (2) it provides qualitative and semi quantitative information of the resolved compounds and (3) it enables the quantification of chemical constituents. Fingerprinting using HPLC and GLC is also carried out in specific cases. In TLC fingerprinting, the data that can be recorded using a High Performance TLC (HPTLC) scanner includes the chromatogram, retardation factor (Rf) values, the colour of the separated bands, their absorption spectra, and λ max and shoulder inflection of all the resolved bands. All of these, together with the profiles on derivatisation with different reagents, represent the TLC fingerprint profile of the sample. The information so generated has a potential application in the identification of an authentic drug, in excluding the adulterants and in maintaining the quality and consistency of the drug.

The fingerprinting of some important secondary metabolites like Saponins, Flavonoids and Alkaloids were obtained by using High Performance Thin Layer Chromatography (HPTLC) Technique. The software of the instrument used is "win CATS and the name of the chromatography is Planer Chromatography". The following fingerprints /chromatograms were obtained.

Identification of Saponins, Flavonoids and Alkaloids were analysed by HPTLC Technique;

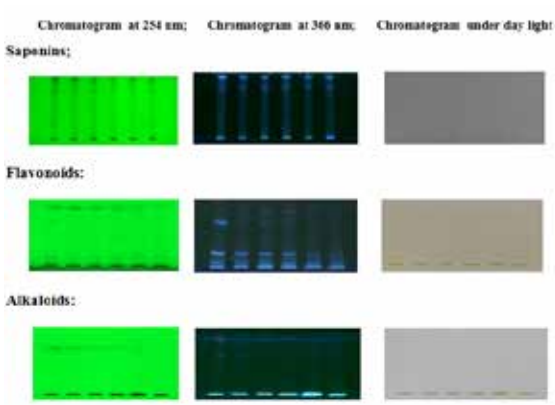
The following parameters were fixed

TLC Chromatograms	: win CATS Planar Chromatography
Instrument	: CAMAG Linomat 5 "Linomat5_080222" S/N 080222(1.00.13)
Executed by	: Anchrom Test Lab Pvt. Ltd. Tuesday, February 26, 2013 5:09:31 PM
Illumination recorded at	: CAMAG Visualizer: 150503
Test performed at	: Anchrom Test Lab P Ltd., Mulund, Mumbai
Day, date & time of test	: February 27, 2013 9:52:43 AM
Exposure mode	: Automatic, digital level: 80 %, Band
Illumination type/correction type	: 254 nm, 366nm & 540nm (There is no band seen at 540nm)
Plate Size	: 20 cm X 10 cm
Spray gas	: Inert Gas
Sample solvent system	: **** Table-2
Adsorbent -coating material, (i.e. Stationary Phase)	: Silica gel GF^{254}
Dosage speed	: 150 nl/s
Predosage volume	: 0.2 µl
Syringe size	: 100 µl
Number of tracks	: 6
Application position Y	: 8.0 mm
Band length	: 8.0 mm
Detection by	: CAMAG TLC Scanner
Position of first track X	: 35.4 mm
Distance between tracks	: 26.0 mm
Scan start pos. Y	: 5.0 mm
Scan end pos. Y	: 85.0 mm
Slit dimensions	: 6.00 x 0.45 mm, Micro
Optimize optical system	: Light
Scanning speed	: 20 mm/s
Data resolution	: 100 µm/step
Wavelength	: 254, 366 & 540 nm
Lamp	: D2
Measurement Type	: Remission
Measurement Mode	: Absorption
Optical filter	: Second order
Detector mode	: Automatic
PM high voltage	: 392 V

Table – 2 (** _The Solvent System differs according to the nature of the ingredient(s)/phytochemical(s) present.)**

S.No	Phytochemical	Suitable Solvent System	Derivatization
01	Saponins (Aq.)	$CHCL_3$: GAA: CH_3OH: H_2O (6.4:3.2:1.2:0.8)	Anisaldehyde Sulphuric Acid
02	Flavonoids (Aq.)	Ethyl acetate: formic acid: GAA: H_2O (10:0.5:0.5:1.3)	Anisaldehyde Sulphuric Acid
03	Alkaloids (Aq.)	Toluene : Ethyl acetate: Di-ethylamine (7:2:1.0)	Dragendorff's Reagent

HPTLC Fingerprinting of some Secondary Metabolites;



Discussion

The study of *Carica papaya* L. leaves found in Northern India was performed upon 6 samples containing 3 types of leaves, i.e. Green, Yellow & Brown from Plain i.e. National Capital Region (NCR) as well as Hilly region like Sunder Nagar (Shimla) in the months of July and August (in rainy season only), dried the healthy leaves after removing their petioles etc.

The significant but some different results amongst 6 samples were obtained. After careful examination and the study of several latest published research papers in reputed International Journals upon the CP leaves and it was observed that none any single ingredient/constituent or factor was found responsible in curing the Dengue/DHF.

None any specific/individual constituent/ingredient or factor was reported till now who is responsible in curing the Dengue, because after careful examination and study of related literature, several other factors were also found responsible in curing the Dengue/DH Fever. After taking the decoction/extraction of CP leaves, some physiological changes occurs in in the patient's body.

In my opinion, some secondary metabolites and some bio-enhancers like Ascorbic acid which is available in CP leaves enhance the physiology in the body of dengue patient & finally, the Basic Metabolic Rate (BMR) increased. In spite of it, the flavonoid quercetin in combination of bio-enhacer has inhibitory activity on NS2B-NS3 protease which is very essential for the viral assembly. Several clinical trials have examined the effect of flavonoid rich foods and beverages on endothelium dependent vasodilation. Endothelial nitric oxide production also inhibits the adhesion and aggregation of *platelets*, one of the first steps in blood clot formation.

There are currently no vaccines available for the prevention/treatment

of Dengue Virus (DEN V) infection. DEN V is a positive stranded encapsulated RNA virus. DEN V is a member of the genus Flavivirus and belongs to the Flaviviridae family. Previous phytochemical analysis reveals so many time by the different techniques the presence of flavonoids like quercetin, alakloids like piperine, glycosides, saponins, tannins, terpenoids like triterpenoids, phytosterols and phenolics were confirmed in an appreciable amount in the leaves of *Carica papaya*. Here in this research paper author also identify most of the phytochemicals present in the leaf of *Carica papaya*, which have mutual and collective effect and responsible for some physiological activities. Ultimately, they inhibit the platelets aggregation and finally enhance the platelet counts in the blood and the patient get cured from Dengue/DHF.

Significance

Dengue is a serious public health problem worldwide. A number of clinical trials have examined the potential for high Flavonoids intakes to decrease various measures of platelet aggregation outside of the body (*ex vivo*) more particularly the quercetin is reported to have inhibitory effect on several viruses. It is also reported that in toxicity study (acute, subcute and chronic toxicity) conducted on *Sprague Dawley* rats administered with *Carica papaya* leaves extract of the Sekaki variant revealed that it was safe for oral consumption. Various previous reports states that the administration of CP leaves extract/decoction exhibits potential anti-dengue activity as indicated by the increased platelet count from 55 X 103/µl to 168 X 103/µl, white blood cells from 3.7 X 103/µl to 7.7 X 103/µl and neutrophils from 46% to 78% in patients with dengue fever.

Cost wise the *Carica papaya* L. Leaves are cost effective, it is very cheap and this plant is cosmopolitan in distribution means it occurs everywhere in the world, a poor person may also afford due to cheaper cost and it is very much available in most of the parts of the world.

Conclusion

It was observed that the bio-enhancers constitute an innovative concept, the research done on the basis of traditional system of Indian Medicine as mentioned in Charaka, Sushruta sanghita by applying the modern techniques. The concept would be useful in decreasing the cost of the drug. The toxicity and other adverse effects are not reported so far. Ultimately, it has a positive influence on the national economy also for the developing countries like India and some poor countries of the world, as desired by WHO also.

In my opinion, CP leaf satisfies all necessary criteria to be considered as a good drug. It is safe, effective, economical, easily available & non-addictive. New drug development technologies are concerned about the economy in the drug development. Drug discovery process has been highly aided by Ayurveda through reverse pharmacology with new means of identifying active compounds and ultimately reduction of drug development cost. The researchers are now aimed at methods of reduction of drug dose also and thus reduction in the drug treatment cost to a wider section of the society including the weaker section of the society.

Now, it has been almost proved that novel drug delivery system of herbal as well as of chemically originated drugs has been used to increase the bioavailability of the compounds or their respective constituents in case of herbal extracts also, which has been mentioned in reported studies related to curcuminoids, silymarin, flavonoids and terpenoids etc.

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