2.0 Introduction

2.1 Overview on Alternative Medicine

Evidence about man’s association with plants or its derived products has been well chronicled since the Neanderthal period (1). Flora with its complex nature of active ingredients and abundance in quantity, geared up to deliver exceptional imprint among medical fraternity (2). Since antiquity Indian medicine, ancient Chinese, Greek & Egyptian population have been used herbal medicines in different forms (3). In recent times numerous researches have focussed in the development of alternative by reconnoitring the plants for the betterment of human life. World Health organization (WHO) termed traditional medicine as “Comprising Therapeutic Practices That Have Been In Reality, Often For Hundreds Of Years, Before The Expansion And Spread Of Modern Medicine And Are Still In Use Today” (4). According to the mandate of WHO resolution AFR/RC50/R3 (2000) the practise of natural products based alternative therapy has been recommended in research & clinical applications, as the resources are found to be abundant (5, 6). WHO statistics cited roughly 3.5 to 4 billion people of the current world population practise 85% of folk medicine for their routine daily needs. It also itemizes nearly 21,000 plants that have been used for its potent therapeutic activity (2, 7).

Every synthetic compound requires 10-15 years with rough estimate cost of € 1 billion to reach clinical practise once obtaining approval from concerned drugs regulatory bodies (8). In spite of approval from preclinical & clinical trials and protracted time progress in screening, some of the synthetic drugs have been withdrawn from the market owing to some undesirable side effects (9). To avert the time & money constraints, several pharmaceutical firms have shifted their focus towards the development of herbal based remedies. In recent times, the perception of developing crops have been shifted for
management of healthy life rather than for foodstuff. Shift in concept leads to the emergence of botanical therapeutics which includes dietary supplements; plant derived recombinant proteins, energy rich food, micronutrient rich foods etc. As a consequence 80% of drugs (antidiabetic, anticancer, immunosuppressive etc.) are found to be of plant origin (10, 11).

Worldwide, herbal medicine has been recognised as one of the potent therapeutic agents for numerous medicinal applications, which subsequently raised the international trade market. By 2050, it has been predicted that global herbal market value will progressed to US $5 trillion from US $62 billion (12). Practise of herbal medicine has been steadily increasing especially among the developing countries in various forms Viz. Indian traditional Medicine, Chinese traditional medicine (13) etc. In the past 10 years, custom of herbal medicine had restored the pharmaceutical business in many developed countries including United States, Australia & Canada (14-16).

From 1990 to ‘97 practise of herbal remedies has massively increased in U.S.A (i.e) up to 380% for major ailments (17). World Health Organisation & World Bank reports suggest that, growth of herbal medicine trade including botanical drug products, medicinal plants & raw materials transpires at an annual rate of 5 - 15% (18). In India, annual herbal medicine trade is estimated as US $10 billion with an exports alone as US $1.1 billion; while in china, annual herbal product export fetches US $3.6 billion (13, 19). Recent reports suggest, India are sole largest exporting country to U.S.A with nearly 50% of their Herbal products (13, 20).

2.2 Herbal Medicine: Back to the Future in path of “Polyherbalism”

In the recent years numerous research studies have focused in the discovery of plant derived drugs in the form of complementary & alternative medicine therapy. Current statistics demonstrates that, nearly 6000 plants in India have been in practise for various therapeutic purposes in the form of traditional or folk or herbal medicine; among this nearly 3000 plants have been scientifically confirmed for their medicinal value (21). In earlier 19th
century, with the assistance of organic methods, the active molecules from plant raw materials were extracted and further instigated in the synthetic methods to improve the yield. As the synthetic drugs were originated for mass production, the usage of herbal materials with active ingredients begun to decline (22). Even though synthetic drugs exhibit robust pharmacological activity, it often ends up with numerous side effects and relatively expensive in market cost. Thus pharmacological activity of plant derived drugs in complementary medicine obliges following categories Viz. identification of selected plant in endangered species list, valid method for extraction & purification of target compound and available concertation of desired active components (2).

As per literature evidence sited in Vedas & Samhitas, Ayurvedic medicine involved herbals as chief components for the development of various therapeutic materials (23). Though numerous bioactive constituents have been well recognised in individual herbs, their negligible levels are inadequate to attain the expected therapeutic effects. However, in Indian system of Ayurvedic medicine, “polyherbalism” is considered to be as one of the dominant therapeutic methodologies for various disorders and has been quoted about 1300 years before in Sharangdhar Samhita (22). Combination of one or more herbs resulted in anticipated pharmacological activity in the form of pharmacodynamics & pharmacokinetic actions & also, it delivers supreme action in low dose with lesser side effects (24).

According to Ethno botanical survey, in Indian system of traditional medicine it has been found that more than 800 individual plants possess anti-hyperglycaemic activity (25, 26). Though individual plants possess anti-hyperglycaemic activity, its negligible level of bioactive constituents hampers to attain the anticipated therapeutic possessions. It is understood that synergistic interfaces among the various constituents in the combination therapy is vital to achieve their therapeutic ability. In our present study, we have selected six
different plants with potent anti-hyperglycaemic activity that had already reported in Indian System of Traditional Medicine for the development of a polyherbal formulation, ADPHF6.

2.3 Review of Literature on Plants used in Development of ADPHF6

2.3.1 *Morinda citrifolia L*

![Image of Morinda citrifolia L](image)

Fig 5: *Morinda citrifolia L*

2.3.1.1 Classification

<table>
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<th>Kingdom</th>
<th>Plantae</th>
<th>Class</th>
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<td><em>Morinda citrifolia</em></td>
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2.3.1.2 Overview about *Morinda citrifolia L*

*Morinda citrifolia* or “Noni” (Fig.5) is expected to be native of Southeast Asia (Indonesia) to Australia. Since antiquity, Noni has been used as a medicinal plant for various illnesses in North-eastern Australia, Polynesia, South and Southeast Asia (27). About 2000 years ago, ancestors of Polynesians are alleged to have brought *morinda citrifolia* plant form Southeast Asia for various medicinal applications and also as a dye for their traditional apparels. Though noni is ethnic to Southeast Asia, it has been cultivated in Caribbean, India, Central and northern America (28, 29). *morinda citrifolia* are commonly named as “Mengkudu” in Malaysia, “Nhau” in Southeast Asia, “Painkiller bush” in the Caribbean, “Indian mulberry”, “Nuna”, or “Ach” in India, and “cheese fruit” in Australia. Its root, stem, leaves & fruits are have diverse medical applications in the form of poultice and infusion.

Products from *morinda citrifolia* are being commercialised in the form of capsules, tea and juice. Noni juice has a wide range of therapeutic activity for increased blood pressure, arthritis, atherosclerosis, menstrual cramps, gastric ulcers etc. In 2003, European Commission has approved Noni juice as Novel food and allowed to be served as Dietary supplement (30). Through numerous oral toxicity and allergenicity studies, European Food Safety Authority (EFSA) has approved the Noni juice as safe food ingredient in 2003 (31). According to Nutrition Business Journal in USA (2005), Noni juice served as dietary supplement, has retained number one position in sales of single herbs & fetched up revenue valued at $250 million (32).

2.3.1.3 Plant Description

The genus *Morinda* [Rubiaceae], comprises nearly 80 species. *Morinda citrifolia*, a small tree measures up to 3-10m high and retain copious elliptical leaves ranging from 5-10 cm (length), 10-40 cm (width). Fruits are globular in shape; size varies from 3-10 cm (wide). Initially during the unripen juncture, fruit appears green in colour and finally turns into
translucent white at ripened stage. Ripened fruits often odour a strong butyric acid like foetid smell. In ripened fruit, pulp is bitter in taste, gelatinous in nature and with hard triangular numerous pits (33).

2.3.1.4 Therapeutic Properties *morinda citrifolia* (fruit)

In recent times, widespread preclinical & clinical investigations have been conducted to validate the pharmacological activity of *morinda citrifolia* fruit. Antioxidant stuff of Noni juice eases strong inhibition of lipid peroxidation during free radical generation as compared with butylated hydroxytoluene (BHT) (34). Polysaccharide rich fractions of noni juice, exhibited antitumor activity in mice model, for Lewis lung carcinoma & sarcoma 180 ascites tumour. It also stimulates cytokines such as TNF- α, IL-1β & IL-10 & IF-γ which will facilitate the tumour cell recognition by cytotoxic effector cells (35). Noni juice further exhibits as a potent anti-inflammatory agent through the selective inhibition of cyclooxygenase enzymes such as COX-1 and COX-2 (36). In STZ induced diabetic study, the *morinda citrifolia* fruit treated Sprague-Dawley rats demonstrate momentous reduction in fasting blood glucose levels from 300 mg/dl (day of Study 3) to 150 mg/dl (day of Study 20) (37). Damnacanthol-3-O-beta-D-primeveroside, a key anthraquinone compound present in noni fruit, responds with hypoglycaemic activity in STZ induced diabetic mice (38). Similarly the body weight of mice measures up to 40% & 25% reduction when treated noni juice along with control & high-fat-diet respectively. Noni juice treated diabetic rat models exemplifies a well reduced hepatocyte fatty degeneration and improved insulin resistance when compared to dietetic group (37).

2.3.2 *Phyllanthus emblica* L.

2.3.2.1 Classification

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</tbody>
</table>

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2.3.2.2 Overview about Phyllanthus emblica L.

*Phyllanthus emblica* or “amla” or “Indian gooseberry” (Fig.6) is native of south-East Asia, and abundantly found in Nepal, Sri Lanka, and Central & Southern India. It has been well adapted to flourish in tropical & subtropical areas (39). Amla has been used in Ayurvedic medicine around 2000 years ago and it is also used as key constituent in Indian indigenous system of medicine. In Ayurvedic literature, Charak samhita and Sushruta Samhita, amla was termed as one of the best rejuvenating herbs among the group of sour fruits (40). To support the previous testimonial, amla has been reported to possess hypoglycaemic, hepatoprotective, antioxidant, hypolipidemic and anti-cancer activities. In
addition, *Phyllanthus emblica* has been documented for antipyretic, analgesic, immunomodulatory, gastro protective and antipyretic activities.

### 2.3.2.3 Plant Description

The genus Phyllanthus (Euphorbiaceae), comprises nearly 550 to 750 species and 10 or 11 subgenera. *Phyllanthus emblica* is a small deciduous tree expected to reach a height of 18 meters. Branches are often flat, pubescent, and 10-20 cm long. Leaves are simple, subsessile with pinnate arrangement and measures to be 8-10 mm long and 2–3 mm broad. Flowers are minute, monoecious, greenish yellow in colour, 0.5-1.5 cm long and also possess widest range of pollen types. Fruits are hard, oblate or spherical in shape, pale greenish yellow in colour and vertical spherical stripes. Size of fruit measured to be 18-25 mm wide and 15-20 mm long. Several phytochemical based reports have confirmed the presence of active ingredients in fruits of *Phyllanthus emblica* which includes vitamin C (200-900 mg/100g), minerals (0.7%), proteins (0.5%) fibre (3.4%) and Fe (1.2 mg/100g) (39).

### 2.3.2.4 Therapeutic Properties

Amla known for rich source of vitamin C improves the natural killer cell & antibody dependant cellular cytotoxicity (41). Oral administration of amla in diabetic rats, resulted in reduction of serum and hepatic mitochondrial thiobarbituric acid-reactive substance levels which eventually leads to delayed aging process (42). Aqueous fruit extract of *Phyllanthus emblica* improves endogenous antioxidant system in hepatocyte cell line (HepG2) and also marks the substantial reduction in levels of Reactive Oxygen Species (ROS) & lipid peroxidation (43). Moreover, supplementation of amla juice marked with heavy metal detoxification in Swiss albino mice against arsenic induced hepatopathy. Reports validates that, administration of *Phyllanthus emblica* fruit extract, significantly reduces the levels of
total cholesterol (TC), low density lipoprotein (LDL), triglyceride (TG) and scripts as potent hypolipidemic agent.

In atherosclerosis condition, oral administration of *Phyllanthus emblica* fruit extract terminates the progression of vascular smooth muscle cell proliferation by inhibiting the oxidized low-density lipoprotein (ox-LDL) (44). Recent clinical studies further confirms that supplementation of amla fruit in diabetic groups marks a major drop in postprandial blood glucose levels (45). In diabetic rats, *Phyllanthus emblica* fruit extract attenuates the neuropathic pain through the alteration of nitrosative stress. Amla also acts as a potent inhibitor for α-amylase & α-glucosidase enzymes which eventually leads to be used for management of Type 2 Diabetes Mellitus (46).

### 2.3.3 *Trigonella foenum-graecum*

![Fig 7: *Trigonella foenum-graecum* (or) Fenugreek](image_url)
2.3.3.1 Classification

<table>
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2.3.3.2 Overview about *Trigonella foenum-graecum*

*Trigonella foenum-graecum* L. or “fenugreek” (Fig.7) is anticipated to be indigenous to Mediterranean region. Trigonella came from Greek word “trigonou” means “triangle”, *foenum-graecum* refers to “Greek Hay” and it has been cultivated in India, China, Africa, Pakistan, Turkey, Spain, and Italy etc. Since ancient times, fenugreek has been used as folk medicine for its therapeutic properties. Reports suggest that application of *Trigonella foenum-graecum* was 1st recorded in ancient Egypt as embalm. It is traditionally Ayurveda & Unani systems as therapeutic agent for anti-oxidant antidiabetic, anti-atherosclerosis, anti-inflammatory and anti-cancer properties (39).

2.3.3.3 Plant Description

Fenugreek is an angiosperm, annual & herbaceous plant; with height expected to reach up to 50 cm. Leaves are oval/triangle, serrated with 3 oblong leaflets. Flowers are pale yellow with 0.8 to 1.8 cm in diameter. Fruits are curved, 3-11 cm long and comprises of 5-20 seeds (4 - 6 cm long). Seeds are yellow in colour, aromatic in taste and rich in nutritional value. It constitutes α-linolenic acid (0.1 g), Riboflavin (0.25 mg), unavailable carbohydrates/
fiber (50 %), β-carotene (2-6 mg), neutral lipids (84.1 %), glycolipids (5.4 %), phospholipids (10.5 %), and saponins (4 to 8 %), alkaloids (1 %) (39).

2.3.3.4 Therapeutic Properties

Generation of Reactive Oxygen Species (ROS) during hyperglycaemic condition will be often increased due to accumulation of excess glucose and fatty acids in adipose tissue (47, 48). Supplementation of *Trigonella foenum-graecum* seed attenuates ROS generation and further improves the endogenous antioxidant activity. Sapogenins one of the key ingredient from fenugreek seeds, exhibits lipid lowering effect through the increased biliary cholesterol excretion and subsequent reduction in the levels of serum triglycerides, total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) (49, 50). Numerous phytochemical investigations authenticate abundant quantity of soluble fibre (30 %) & insoluble fibre (20 %) in *Trigonella foenum-graecum* seeds that facilitates reduced post prandial glucose absorption. These fibres defends the colon mucus membrane by binding with toxins exists in the diet (51). Interestingly, Galactomannan a soluble fibre exists in fenugreek seed has been reported for reduced uptake of bile salts in intestine and absorption of starch in human body (52).

According to Broca *et al.* (2000) 4-hydroxyisoleucine, isolated from fenugreek seeds triggers the insulin secretion from pancreatic beta cells & thereby acts as an insulinotropic agent during Type 2 diabetes mellitus condition (53). Clinical study confirms that *Trigonella foenum-graecum* seeds exhibit hypoglycaemic effect through the inhibition of the carbohydrate hydrolysing enzymes such as α-amylase & α-glucosidase and prevents Type 2 diabetes mellitus related secondary complications (54). Reports from double blind clinical study concludes that administration of fenugreek in diet responded with improved glucose tolerance and polyuria condition in type 2 diabetic affected groups (55). In skeletal muscles, insulin stimulation is prerequisite in glucose uptake through the translocation of glucose
transporter-4 (GLUT-4). But during diabetic condition, insulin deficiency resulted in retention of GLUT-4 and which further leads to elevated blood sugar level. In experimental diabetic animal models, oral administration of fenugreek seed extract reverses the insulin action and improves the translocation GLUT-4 protein (56).

2.3.4 *Psidium guajava*

2.3.4.1 Classification

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</tr>
</tbody>
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![Fig 8: Psidium guajava or Guava](image)
2.3.4.2 Overview about *Psidium guajava*

*Psidium guajava* (Fig.8) is predicted to be native in America from Mexico to Brazil & covers all over the Africa, Asia (India), Europe, South America. *Psidium guajava* is commonly named as *goyave* or *goyavier* (French), *banjiro* (Japan), *guyaba* or *guave* or *surinamese* or *goeajaaba* (Dutch), *guaiaba* (Brazil), *goiaba* or *goaibeira* (Portuguese), *guava* or *kuawa* (Hawaii). Guava has been habitually used in pre-Columbian times (57). In Egypt, since antiquity *Psidium guajava* has been used in folk medicine for the management of diabetes mellitus, obesity, hypertension, gastroenteritis, diarrhoea etc. Traditional practice of guava was well documented among the ethnic groups of Mexican Indians, Maya, Zapotec etc for digestive problems. Guava leaves has been applied externally for numerous anti-inflammatory diseases. *Psidium guajava* fruit has been used as an alternative medicine in Brazil for inflamed mucous membrane, ulcers, anorexia etc. (58).

2.3.4.3 Plant Description

*Psidium guajava* is adapted to grow in all tropical & subtropical conditions. It is small tree, with height measuring up to 10 meters. Bark appears as thin, patchy and smooth. Leaves are oval in shape, with short petiole, prominent pinnate veins and measures up to 5-15 cm long. Flowers are 2 cm long and whitish in colour with numerous stamens. Fruits are fleshy (5 cm), with edible mesocarp bearing numerous tiny, hard seeds. Guava fruits comprise 84.9 % water content, 0.88 % proteins, 0.53% fats & 13.2 % carbohydrates. Fruits are known for high nutritional value such as ascorbic acid (100 mg/100 g), riboflavin (0.03-0.04mg/100g), thiamine 0.046mg/100g, iron (0.30-0.70mg/100g), calcium (9.1- 17mg/100g), fibre (2.8-5.5g/100g), Vitamin A & B3 (200-400 & 40 I.U) (59).

2.3.4.4 Therapeutic Properties

Guaijaverin and quercetin from guava leaves acts as a potent antimicrobial and antiplaque agent by impeding the growth of *S. mutans* and *Staphylococcus aureus* & further...
reins the development of dental plaque. Studies suggest that lectin from *Psidium guajava* fruit prevents the adhesion of *E.coli* to intestinal wall and thereby reduces the incidence of diarrhoeal infection (60). Guava’s peel & pulp extract materialises as a potent antioxidant agent by inhibiting copper induced low density protein LDL oxidation. In Streptozotocin (STZ) induced diabetic rat study *Psidium guajava* fruit showed a significant reduction blood glucose level (152 mg/dL) and improves the concentration of insulin levels (90%) & reinstates the body weight. STZ known to act specifically and destroys the pancreatic β cells through DNA strand breaks which in turn end up in decreased insulin production. However, *Psidium guajava* fruit have remarkably reduces the DNA strand breaks in pancreatic cells through the attenuation of lipid peroxidation.

In addition, Guava fruit extract unveils its effective anti-oxidant property by improving the endogenous antioxidant enzymes such as SOD, CAT, and GPx which are essential for the inhibition of free radicals mediated destruction in pancreatic β cells (61). In diabetic mice model, aqueous fruit extract of *guava*, demonstrates an anti-glycative activity and further recovers the renal functions in kidney. Moreover, surplus production of cytokines is believed to enhance the diabetic complications. Administration of fruit extract augments (2%) the inflammatory cytokines such as IL-6 and TNF-α, IL-1β which in response improves the renal function (62).

### 2.3.5 *Syzygium cumini* (L.) Skeels

#### 2.3.5.1 Classification

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2.3.5.2 Overview about *Syzygium cumini* (L.) Skeels

“*Syzygium cumini* (L.) Skeels” or “jamun” or “Indian black berry” (Fig.9) is anticipated to be native of tropical region and widely distributed in India, Sri Lanka & Australia. Jamun has been established for greater commercial value in Florida, Israel, California & Algeria. From the time of ancient times in Madagascar, traditional naturopathies have used jamun plant as an operational therapy for diabetes mediated complications (63). To substantiate this report, numerous studies (1960-70’s) have been conducted with the crude extracts of jamun plant in experimental models and confirmed its anti-hyperglycaemic activity. It has been primarily used for anti-oxidant and anti-inflammatory activities since ancient times. Moreover it possesses a wide range of therapeutic actions Viz., anti-diarrhoeal, free radical scavenging, cardioprotective, anti-cancer, antimicrobial, strangury,
hepatoprotective properties. Notably in folk medicine, jamun seed used as potent agent for blood purification (64).

2.3.5.3 Plant Description

*Syzygium cumini* (L.) Skeels belongs to family Myrtaceae have been used throughout the tropical & subtropical regions for its rich medicinal value. It is large, foliaceous tree with brownish bark. Leaves are oval, auxiliary or terminal & measuring up to 6-12 cm. Flowers are arranged in clusters (10-40 in no’s) and oblong in shape. Fruits are oblong in shape, dark-purple in colour, measuring 1.5 to 3.5 cm long. Fruits are abundant in phytochemical constituents Viz., ascorbic acid (5.70-18 mg), riboflavin (0.01 mg), protein (0.70-0.13 g), vitamin A (80 I.U.), iron (1.62 mg), calcium (8.30-15 mg), thiamine (0.01-0.03 mg), potassium (55 mg), magnesium (35 mg), sodium (6.20 mg), crude fiber (0.90 g) per 100 g of edible portion (63).

2.3.5.4 Therapeutic Properties

Aqueous extract of *Syzygium cumini* validates a distinct antiallergic property through the inhibition of C48/80 induced paw edema & further prevents the eosinophil accumulation in Swiss mice model (65). Glycoside jambolin & ellagic acid present in jamun seeds, plays an active role in terminating the conversion of complex starch into active sugar molecule & further reduces blood pressure (34.6%). Oleanolic acid from jamun extract reduces the action of free radicals (60-90%) and prevents the further development of atherosclerosis in diabetic individuals (66). Oral supplementation of LH II from jamun seeds remarkably decrease the levels of HDL, triglycerides & serum total cholesterol in diabetic rabbit models (67). Histological reports suggest that aqueous extract jamun attributes in restoration of the structural pancreatic β-cells & further enhances the stimulation of insulin secretion from the β-cells of islet of Langerhans (64). Interestingly, extracts of *Syzygium cumini* exhibits
significant activity for \( \alpha \)-glucosidase enzyme inhibition and improves the glucose tolerance in Goto-Kakizaki (GK) rat model.

Supplementation of jamun fractions (200 & 400 mg/kg) displayed a significant decrease in blood sugar level in STZ induced diabetic model (68). Further in mixture with banana extract, jamun aqueous extract reinstates the functions of hepatic hexokinase and glucose-6-phosphatase in diabetic rats (69). To the added benefit, aqueous extract of jamun seeds deteriorates the N-acetyl-\( \beta \)-D-glucosaminidase (NAG) & lipid peroxidation levels due to methylmercury induced toxicity in rat model (70). *Syzygium cumini* had been in used numerous herbal antidiabetic formulations such as Diabeta, Dianex, Diabecon, and Dihar etc and has produced effective hypoglycaemic activity (63).

### 2.3.6 *Momordica charantia* L

![Fig 10: Momordica charantia](image-url)


2.3.6.1 Classification

Kingdom : Plantae
Subkingdom : Viridiplantae
Infrakingdom : Streptophyta
Superdivision : Embryophyta
Division : Tracheophyta
Subdivision : Spermatophytina
Class : Magnoliopsida
Superorder : Rosanae
Order : Cucurbitales
Family : Cucurbitaceae
Genus : Momordica L.
Species : Momordica charantia L.

2.3.6.2 Overview about *Momordica charantia* L.

"*Momordica charantia*” or “bitter melon” or “bitter gourd” (Fig.10) is anticipated to be indigenous to East Africa, South America, India, and Caribbean. Bitter gourd grows vastly in tropical regions & used in folk medicines for its numerous medicinal properties (71). From ancient times, *Momordica charantia* has been predominantly used for diabetic complications as a hypoglycaemic agent. In Latin America, & Africa, it has been termed as “vegetable insulin” since some of the components believed to be resemblance of animal inulin molecule (72). In traditional system of alternative medicine, *Momordica charantia* has been used for wide range of therapeutic applications Viz., rheumatism, pneumonia, anthelmintic, jaundice, scabies, kidney disorders etc., (73).

2.3.6.3 Plant Description

*Momordica charantia* is widely cultivated vegetable crop in Asia & South America. It is herbaceous in nature, flowering vine with juvenile stems & leaves. Leaves are simple, palmate (5-7 lobes), alternate in arrangement & grows up to 5 m long. Flowers are staminate, calyx & corolla are lobed, and yellow in colour. Fruits are ovoid, spindle shaped measures up to 3-5 cm. It is hollow in cross section, with layer of flesh covering the seed cavity. Initially, unripe fruits are tender & green in colour, and changes into orange & mushy at ripen stage.
Momordica charantia comprises water (93.2 %), protein (18.02 %), lipids (0.76 %); while its seed possess eleostearic acid (63-68 %), and stearic acid (22-27 %) (74).

2.3.6.4 Therapeutic Properties

Momordica charantia comprises numerous biologically active molecules or substances which are reported for vast therapeutic value. To validate this report, molecules such as Charantins, momordin Ic, oleanolic acid, 3-O-monodesmoside isolated from bitter gourd fruit have been reported for hypoglycaemic activity (73). MRK29 (Mol.Weight 28.6 kD) from bitter gourd fruit exhibited anti-HIV activity through the inhibition of p24 expression, a viral coat protein (82%) in HIV-infected cells (75).

According to Bailey et al., (1985) fruit, pulp, seed & leaves of Momordica charantia demonstrated the anti-hyperglycaemic activity in streptozotocin induced models (76). Follow up studies confirms the hypoglycaemic activity comprises through the sequence of actions Viz., recovery of the β cell action, stimulation in the release of insulin molecule, increased GLUT4 transporter proteins, and stimulation of glucose-6-phosphate dehydrogenase activity (77). Numerous preclinical studies substantiates that, Momordica charantia deters the development of diabetic complications such as insulin resistance, nephropathy, neuropathy and cataract (73).

2.4 Materials & Methods

2.4.1 Authentication of Plants

Trigonella foenum-graecum L, Syzygium cumini (L) skeels, Momordica charantia L, Phyllanthus emblica L. and Psidium guajava L were collected from medicinal farm Frontier Mediville (Elavur, Gummidipoondi, India). Morinda citrifolia L was collected from World Noni Research Foundation (Chennai, India).
Herbarium specimens of six individual plants were identified and authenticated by Prof. P Jayaraman, Director, Institute of Herbal Science Plant Anatomy Research Centre, Chennai, Tamilnadu. The Herbarium specimen & Authentication Certificate of individual Plant material has been provided in Figure 11-22. The plant names with Species, Families and Parts used as well as their therapeutic effects is listed in Table 3.

2.4.2 Development of ADPHF6, Polyherbal Formulation

In our present study for enzyme inhibition assay and phytochemical evaluation, we have pursued lyophilization and aqueous extraction process as an alternative of shear dry & solvent extraction method, since former renders less denaturing activity to peptides or proteins present in the ADPHF6 extract.

The water soluble proteins exists in natural sources possessing inhibitory activity for α-amylase and α-glucosidase enzymes, can be efficiently condensed to a fine powder in stable form by involving freeze drying and aqueous extraction method (78, 79). The above mentioned parts of six anti-hyperglycaemic plants were collected and processed within 2 hr at sterile conditions. All the individual parts are finely crushed and lyophilized or freeze dried. For lyophilization, samples were frozen at -50\(^\circ\) C in spherical trays of 200mm dia for 6-7 hr.

The Prefrozen samples were exposed to sublimation with a standard condition of condenser at -50\(^\circ\) C and a rotary vacuum pump to achieve a vacuum of <10 Pa. The final freeze dried samples were confirmed for minimal moisture (1-2%) content using a temperature probe. The freeze dried powder was stored in air tight container at room temperature for further experimental analysis. Lyophilized powder from individual plants were mixed in precise proportion (Table 3) and soaked in sterile milliQ water for overnight incubation and filtered. ADPHF6 extract was prepared in fresh batch for each experimental trial.
<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Parts Used</th>
<th>Collection of Plants</th>
<th>Quantity Used</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Morinda citrifolia L</em></td>
<td>Rubiaceae</td>
<td>Fruit</td>
<td>World Noni Research Foundation, Chennai, Tamil Nadu</td>
<td>10 mg</td>
</tr>
<tr>
<td><em>Trigonella foenum-graecum L</em></td>
<td>Fabaceae</td>
<td>Seeds</td>
<td>Chennai, Tamil Nadu</td>
<td>30 mg</td>
</tr>
<tr>
<td><em>Syzygium cumini (L) Skeels</em></td>
<td>Myrtaceae</td>
<td>Seeds</td>
<td>Medicinal Farm, Frontier, Mediville, Gummidiipoondi, Tamil Nadu</td>
<td>30 mg</td>
</tr>
<tr>
<td><em>Momordica charantia L</em></td>
<td>Cucurbitaceae</td>
<td>Fruit</td>
<td>Medicinal Farm, Frontier, Mediville, Gummidiipoondi, Tamil Nadu</td>
<td>10 mg</td>
</tr>
<tr>
<td><em>Phyllanthus emblica L.</em></td>
<td>Euphorbiaceae</td>
<td>Pulp</td>
<td>Medicinal Farm, Frontier, Mediville, Gummidiipoondi, Tamil Nadu</td>
<td>10 mg</td>
</tr>
<tr>
<td><em>Psidium guajava L.</em></td>
<td>Myrtaceae</td>
<td>Fruit</td>
<td>Chengalpattu, Tamil Nadu</td>
<td>10 mg</td>
</tr>
</tbody>
</table>
Fig 11: *Morinda citrifolia* L. Herbarium Specimen
Fig 12: *Morinda citrifolia* L Authentication Certificate
Fig 13: *Trigonella foenum-graecum* L Herbarium Specimen
Fig 14: *Trigonella foenum-graecum* L. Authentication Certificate
Fig 15: *Syzygium cumini* (L) Skeels L Herbarium Specimen

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Fig 16: *Syzygium cumini* (L) Skeels L Authentication Certificate
Fig 17: *Momordica charantia* Herbarium Specimen
AUTHENTICATION CERTIFICATE

Based upon the Organoleptic/macroscopic/microscopic examination of fresh/market sample, it is certified that the specimen given by Devanand S., Ph.D. Research Scholar, [Affiliated to University of Madras] is identified as below:

Binomial: **Momordica charantia** L. var. charantia Wight, I. C.

Family: **Cucurbitaceae**

Synonym(s):

Regional names: Tamil: Pavakai, Bitter gourd

Reg. No. of the certificate: **PARC 2014/2034**

References: Nair, N.C & Henry, A.N. Flora of Tamil Nadu, India


Henry, A.N. et al.


Date: 05.09.2014
Fig 19: *Phyllanthus emblica* Herbarium Specimen
Fig 20: Phyllanthus emblica Authentication Certificate

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Fig 21: *Psidium guajava* L Herbarium Specimen
AUTHENTICATION CERTIFICATE

Based upon the Organoletic/macroscopic/microscopic examination of fresh/market sample, it is certified that the specimen given by Devanand S. Ph.D. Research scholar, [Affiliated to University of Madras] is identified as below:

Binomial: **Psidium guajava** L.

Family: **Myrtaceae**

Synonym(s):

Regional names:

Reg. No of the certificate: PARC 2014 2031

References:


Date: 05.09.2014

(Prof. P. Jayaraman, Ph.D. Director, Institute of Herbal Science, PLANT ANATOMY RESEARCH CENTRE, No.4-B Street, Sakthi Nagar, West Tambaram, Chennai-600 045. Ph: 044-22263236, +919444385998. Email: herbauthentication.com)

Fig 22: Psidium guajava L Authentication Certificate
2.4.4 Organoleptic evaluation

The organoleptic evaluation of polyherbal formulation was evaluated with minor modifications (80). Organoleptic evaluation discusses the evaluation of formulation by colour, odour, taste, texture, appearance etc.

2.4.5 Physicochemical evaluation

2.4.5.1 Moisture Content

Moisture content of ADPHF6 was determined by loss on drying (LOD) using Karl Fischer method (81). The finely lyophilized polyherbal formulation powder was allowed passed through #40 and retained on #120 mesh strainer. 10 g of filtered ADPHF6 powder was dried at 105°C for 5 hours in tray drier and weighed. The drying process was continued at one-hour interval until difference was not more than 0.25% and 0.01 g.

2.4.5.2 Bulk Density & Tapped density

Polyherbal formulation of about 50 cm³ powder has been filtered through a #20 sieve was carefully introduced into a 100 mL graduated cylinder (81). The cylinder was placed at 2-s intervals on a wooden surface 3 times from a height of 1 in. The initial volume was noted and the sample has been tapped until no further reduction in volume was noted. The bulk and tapped density was then measured by dividing the weight of the sample (g) to the final volume in cm³ of the ADPHF6 sample.

2.4.6 Determination of pH

The pH of freshly prepared ADPHF6 polyherbal herbal formulation in 1% w/v of aqueous soluble portion was determined using glass electrode pH meter.

2.4.7 Determination of Ash values
2.4.7.1 Ash Content

5 g of lyophilized powder of polyherbal formulation was placed in pre heated silica crucible (81). Selected herbal material has been spread in an even layer and ignited gradually kept in a muffle furnace at 400-500°C until the change in white color. Material was cooled in a desiccator and the content of total ash was calculated in mg/g of air dried test material.

2.4.7.2 Acid insoluble Ash

The total ash of polyherbal formulation was boiled with 25 ml of 2 M Hydrochloric acid for 5 minutes in crucible covered with watch glass (81). The insoluble matter of herbal material was collected on a fresh filter paper and washed with hot water. The ash content was then dried and weighed immediately for insoluble ash content.

2.4.5 Screening of Phytochemical Contents in ADPHF6

2.4.5.1 Phytochemical screening by Qualitative Analysis (82)

2.4.5.1.1 Test for Alkaloids

Mayers test: To the one gram powder of ADPHF6, 3ml of ammonia solution and 2ml of chloroform was added. To the filtrate, 3ml of Mayer’s reagent was added. Appearance of Pale precipitate indicates the presence of alkaloids.

2.4.5.1.2 Test for Flavonoids

To the one ml of ADPHF6 aqueous solution, few drops of dilute sodium hydroxide solution was added. After the development of yellow colour in the reaction, few drop of concentrated sulphuric acid was added. Disappearance of yellow colour indicates the presence of flavonoids.
2.4.5.1.3 Test for Phenols and Tannins

**Ferric Chloride Test:** Few drops of 10% Ferric chloride were added to 300μl of ADPHF6 aqueous extract. Appearance of Blue or Green colour precipitate, indicates the presence of Phenol and Tannin.

2.4.5.1.4 Test for Steroids

**Liebermann Burchard reaction:** To 5ml of the ADPHF6 aqueous extract, 2 ml of acetic anhydride and 2 ml conc. Sulphuric acid was added. Change of colour from violet to blue indicates the presence of steroids.

2.4.5.1.5 Test for Terpenoids

**Salkowski Test:** 5 mg of ADPHF6 powdered sample was dissolved in 2ml of chloroform. Further 1ml of acetic anhydride was added to the mixture and kept for incubation at room temperature for 2 minutes. 1ml of conc. sulphuric acid was added along the sides of the test tube. Development of reddish violet colour precipitate indicates the presence of terpenoids.

2.4.5.1.6 Test for Anthraquinones

**Borntrager’s Test:** To 300μl of ADPHF6 aqueous extract, 1ml of benzene and 10% ammonia was added. Appearance of formation of pink, red or violet colour indicates the presence of anthraquinones.

2.4.5.1.7 Test for Saponins

**Froth test:** 0.5 g of the ADPHF6 extract was dissolved in 2ml of distilled water. The mixture was vigorously shaken and observed for the stable froth persistence. Appearance of a foam layer indicates the presence of saponins.

2.4.5.1.8 Test for Reducing Sugar

To 1ml of ADPHF6 aqueous extract, few drops of Molisch’s reagent were added and kept in water bath for 30 minutes. Appearance brick red coloured precipitate indicates the presence of reducing sugar.
2.4.5.1.9 Test for Cardiac Glycosides

**Keller Kiliani Test:** To the 300μl of ADPHF6 aqueous extract, 1ml of Acetic acid followed by the addition of 300μl of 10% Ferric Chloride. Few drops of Concentrated Sulphuric acid were added along the sides of the test tubes. Appearance of brownish ring and green blue precipitate indicates the presence of cardiac glycosides.

2.4.5.1.10 Test for Lipids and Fat

100 mg of ADPHF6 powder sample was rubbed on a fresh filter paper. Appearance of a permanent translucent strain indicates the presence of lipids and fat.

2.4.6 Quantitative Analysis of Phytochemical constituents

2.4.6.1 Estimation of Total Phenolic content (83)

2.4.6.1.1 Requirements

a) Ethanol : 40%
b) Folin-Ciocalteu (FC) Reagent & Milli Q water : 1:1
c) Sodium carbonate : 20%
d) Quercetin (Standard Stock Solution) : 1 mg/ml in Ethanol
e) Quercetin Working Concentration : 0.1mg/ml in Ethanol

2.4.6.1.2 Procedure

Total phenolic content in aqueous extract of ADPHF6 was evaluated with modified Folin-Ciocalteu method. Gallic acid was used as reference standard for total phenolic estimation. ADPHF6 sample (0.2-1mg/ml) was added in different aliquots of 20 μl to 100 μl in graduated tubes. To each tube 0.5 ml of 1:1 FC reagent was added and incubated for 10 min at room temperature. Further, 2.5 ml of saturated sodium carbonate solution was added and incubated at room temperature for 30 min. Phenols in ADPHF6 extract were reduced by FC reagent and the absorbance was measured at 750 nm (UV-1800, Shimadzu UV spectrophotometer).
Reaction mixture involving FC reagent and Na\textsubscript{2}CO\textsubscript{3} solution alone served as blank. Total phenol content was expressed in terms of gallic acid equivalents (GAE) using the equation \( T = C \times V / M \) (\( T = \) Total Phenolic Content (mg/g) of extract as gallic acid equivalents, \( C = \) Concentration of Gallic acid established from the calibration curve, \( V = \) Volume of the extract solution in ml, \( M = \) Weight of extract in grams).

### 2.4.7.2 Estimation of Total Flavonoid content (84)

#### 2.4.7.2.1 Requirements

- **a)** Ethanol : 40%
- **b)** Aluminium Chloride : 10% AlCl\textsubscript{3} 10% Methanol
- **c)** Sodium Potassium Tartrate : 0.282mg in 10ml of Distilled Water
- **d)** Quercetin (Standard Stock Solution) : 1 mg/ml in Ethanol
- **e)** Quercetin Working Concentration : 0.1mg/ml in Ethanol

#### 2.4.7.2.2 Procedure

Total flavonoid content in aqueous extract of ADPHF6 was evaluated with minor modifications from Cameron GR., 1943 (84). Quercetin was used as reference standard. ADPHF6 sample (0.2-1mg/ml) was added in different aliquots of 20 \( \mu \)l to 100 \( \mu \)l in graduated tubes. 0.5 ml of 10% AlCl\textsubscript{3} in 10% methanol and 0.5 ml sodium potassium tartarate were added and the final volume was made up to 3 ml with water. The reaction mixture was incubated for 30 minutes in the dark and the absorbance was read at 415 nm (UV- 1800, Shimadzu UVspectrophotometer). AlCl\textsubscript{3} and sodium potassium tartarate alone served as blank. The total flavonoid content in the ADPHF6 extract was expressed in terms of Quercetin equivalents.

### 2.4.8 In Vitro Antioxidant Activity

#### 2.4.8.1 DPPH Radical Scavenging Assay (85, 86)
2.4.8.1.1 Reagents Required/Requirements

a) Methanol : 95%
b) 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) : 0.006% in 10% Methanol.
c) Ascorbic Acid (Standard Stock Solution) : 1mg/ml in Distilled Water (Stock)
d) Ascorbic Acid (Standard Working Concentration) : 0.1mg/ml

2.4.8.1.2 Procedure

The Free radical scavenging activity of the ADPHF6 aqueous extract was measured with modified procedure adapted from Khan et al., 2013 & Mensor et al., 2001. The reaction mixtures comprised of 1 ml of 0.1mM 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) in 95% methanol, ADPHF6 aqueous extracts at various concentrations ranging from 20-100 mg/ml (w/v) and final volume was made up to 2 ml with 95% methanol. The reaction mixtures were vortexed thoroughly and allowed in dark condition at room temperature for 30 min. The bleaching of stable free radical (DPPH) by ADPHF6 extract was determined by measuring the absorbance at 515 nm (UV- 1800, Shimadzu UVspectrophotometer). Ascorbic acid (0.1 mg/ml) served as positive control; 95% methanol & DPPH served as blank. Percentage of DPPH radical scavenging activity (IC_{50}) was calculated by the following equation,

\[
\% \text{ Inhibition} = \left\{ \frac{\text{Absorbance of Control} - \text{Absorbance of Extract}}{\text{Absorbance of Control}} \right\} \times 100
\]

2.4.8.2 Hydrogen Peroxide (H_2O_2) Radical Scavenging Activity (87)

1.4.8.2.1 Requirements

a) Hydrogen peroxide : 40 mM
b) Phosphate buffer : 50 mM
c) Ascorbic Acid (Standard Stock Solution) : 1mg/ml
d) Ascorbic Acid (Standard Working Concentration) : 0.1mg/ml

2.4.8.2.2 Procedure

The scavenging ability of ADPHF6 aqueous extract on Hydrogen peroxide was measured with modified procedure adapted from Gulcin et al., 2004. The reaction mixture comprised of 0.5 ml of 40 mM hydrogen peroxide, ADPHF6 extracts at varying concentrations from 20-100 mg/ml (w/v) and final volume was made up to 3 ml with 50 mM Phosphate buffer, pH 7.4. The reaction mixture was vortexed thoroughly and allowed at room temperature for 10 min. The absorbance was measured at 230 nm (UV-1800, Shimadzu UVspectrophotometer). Ascorbic acid (0.1 mg/ml) served as positive control. Hydrogen peroxide & Phosphate buffer served as blank. Percentage of hydrogen peroxide scavenging activity was determined and calculated by the following equation,

\[
\% \text{ Inhibition} = \left( \frac{\text{Absorbance of Control} - \text{Absorbance of Extract}}{\text{Absorbance of Control}} \right) \times 100
\]

2.4.8.3 Metal Chelating Activity (88)

2.4.8.3.1 Requirements

a) Ferric Chloride (FeCl\(_2\)) : 2mM
b) Ferrozine : 5mM
c) Ethylenediaminetetraacetic acid [EDTA] : 1mg/ml in Distilled Water (Stock)
d) Ethylenediaminetetraacetic acid [EDTA] : 0.1mg/ml (Working Concentration)

2.4.8.3.2 Procedure

The chelating activity of ADPHF6 aqueous extract for ferrous ions Fe\(^{2+}\) was determined with modified procedure adapted from Loizzo et al., 2012 & Dinis et al., 1994. The reaction mixture comprised of 0.1 ml of 2 mM FeCl\(_2\), ADPHF6 extracts in deionized water at different concentrations ranging from 20-100 mg/ml (w/v), 0.2 ml of 5 mM ferrozine and final volume was made up to 1 ml with deionized water. The reaction mixture was vortexed...
thoroughly and allowed at room temperature for 10 min. Development of ferrozine-Fe$^{2+}$ complex was determined by measurement of absorbance at 562 nm (UV-1800, Shimadzu UV spectrophotometer). EDTA (0.1 mg/ml) was served as positive control; FeCl$_2$ & ferrozine served as blank. Chelating activity of the ADPHF6 extract for Fe$^{2+}$ (IC$_{50}$) was calculated by the following equation,

\[
\% \text{ Inhibition} = \left\{ \frac{(\text{Absorbance of Control} - \text{Absorbance of Extract})}{\text{Absorbance of Control}} \right\} \times 100
\]

2.4.9 Antidiabetic Screening Assay

2.4.9.1 Inhibition Assay for α-Amylase Activity (78)

2.4.9.1.1 Reagents Required

a) Sodium phosphate (NaPO$_4$) buffer : 20 mM
b) Sodium chloride (NaCl) : 6 mM
c) Porcine Pancreatic α-amylase : 0.05 units
d) Starch : 0.5%
e) 3, 5-dinitrosalicylic acid (DNSA) : (96 mM 3,5-dinitrosalicylic acid, 5.31 M sodium potassium tartrate in 2 M NaOH)
f) Acarbose : 0.1 mg/ml

2.4.9.1.2 Procedure

Screening of polyherbal formulation (ADPHF6) for potent α-amylase inhibition was performed using DNSA (3, 5-dinitrosalicylic acid) method adapted from P et al., 2011. Porcine Pancreatic α-amylase (SRL Pvt. Ltd., (Mumbai, India) was dissolved in 20 mM sodium phosphate buffer (pH 6.9) to give a concentration of 0.05 units. The reaction mixtures comprised of 500 μl with 20 mM sodium phosphate buffer, pH 6.9 (containing 6 mM sodium chloride), 0.05 units of PPA solution and ADPHF6 aqueous extracts at various concentration ranging from 0.1-1.5 mg/ml (w/v) were incubated for 10 min at 37°C. After pre-incubation, 250 μl of 0.5% (v/v) starch solution (as substrate) was added to each reaction mixture and
incubated for 15 min at 37°C. After the incubation period, the reaction was terminated by adding 1.0 ml DNSA reagent (HiMedia Laboratories, Mumbai, India). After thorough mixing, the samples were placed in boiling water bath for 10 min. All the samples were cooled to room temperature and the absorbance was measured at 540 nm (Shimadzu UV-VIS 1800 spectrophotometer; Shimadzu Corporation, Kyoto, Japan). Acarbose served as Positive control; Reaction mixture with PPA served as control.

2.4.9.2 Inhibition Assay for α-Glucosidase Activity (90)

2.4.9.2.1 Reagents Required

a) Sodium Phosphate (NaPO₄) buffer : 0.1M
b) Sodium chloride (NaCl) : 6 mM
c) Sodium Carbonate (Na₂CO₃) : 0.1M
d) α-glucosidase : 0.1 units
e) p-nitrophenyl α-D glucopyranoside : 2 mM
f) Acarbose : 0.1 mg/ml

2.4.9.2.2 Procedure

The α-Glucosidase inhibition assay was performed in microtiter plate with modified procedure adapted from kim et al., 2005. The reaction mixtures comprised of 170 µl of 0.1M Sodium Phosphate buffer, pH 6.9 (containing 6mM NaCl), 0.1 units of α-glucosidase and ADPHF6 aqueous extracts at various concentration from 0.1-1.5 mg/ml (w/v) were incubated for 10 min at 37°C. After pre-incubation, 70 µl of 2 mM p-nitrophenyl α-D glucopyranoside, as substrate) in 0.1M Na₂PO₄ buffer was added to each reaction mixture and incubated at 37°C for 5 min. The reaction was terminated by adding 70 µl of 0.1M Na₂CO₃. Absorbance was measured at 405 nm (Bio-Rad microplate reader; Bio-Rad Laboratories, California, USA). Acarbose served as Positive control; Reaction mixture with α-glucosidase served as
\[
\% \text{Inhibition} = \left( \frac{\text{Absorbance of Control} - \text{Absorbance of Extract}}{\text{Absorbance of Control}} \right) \times 100
\]

2.5 Results

2.5.1 Organoleptic & Physicochemical Properties

Organoleptic analysis (Table 4) of polyherbal formulation was observed for buff colour, intense pleasant odour, slightly bitter in taste, fine powder in texture and pH of 5.9-6.0. ADPHF6 polyherbal formulation has been analysed for physicochemical properties are listed in Table 5. Bulk and Tapped density was measured as 0.268±0.72 g/cc, 0.379±0.03 g/cc respectively. The formulation also exhibited total ash content 3.28 ± 0.02 % (by Mass), Sulphate Ash 2.28 ±0.01 % (by Mass), Nitrated Ash 2.50±0.10 % (by Mass), Acid Insoluble Ash 2.02±0.32 % (by Mass), Water Insoluble Matter 2.27±0.11 % (by Mass), Moisture Content (Loss on drying) 8.31± 0.012 % (by Mass).

Table 4: Organoleptic properties of ADPHF6 polyherbal formulation

<table>
<thead>
<tr>
<th>S. No</th>
<th>Property</th>
<th>Characteristic Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Colour</td>
<td>Buff</td>
</tr>
<tr>
<td>2.</td>
<td>Odour</td>
<td>Intense pleasant odour</td>
</tr>
<tr>
<td>3.</td>
<td>Taste</td>
<td>Slightly bitter</td>
</tr>
<tr>
<td>4.</td>
<td>Appearance/Texture</td>
<td>Fine Powder</td>
</tr>
<tr>
<td>5.</td>
<td>pH at 4-8°C</td>
<td>5.9-6.0</td>
</tr>
</tbody>
</table>
Table 5: Physicochemical Analysis of ADPHF6 Polyherbal formulation

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Mean</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Bulk density</td>
<td>0.268 ± 0.72</td>
<td>g/cc</td>
</tr>
<tr>
<td>2.</td>
<td>Tapped density</td>
<td>0.379 ± 0.03</td>
<td>g/cc</td>
</tr>
<tr>
<td>3.</td>
<td>Ash Content</td>
<td>3.28 ± 0.02</td>
<td>% (by Mass)</td>
</tr>
<tr>
<td>4.</td>
<td>Sulphate Ash</td>
<td>2.28 ± 0.01</td>
<td>% (by Mass)</td>
</tr>
<tr>
<td>5.</td>
<td>Nitrated Ash</td>
<td>2.50 ± 0.10</td>
<td>% (by Mass)</td>
</tr>
<tr>
<td>6.</td>
<td>Acid Insoluble Ash</td>
<td>2.02 ± 0.32</td>
<td>% (by Mass)</td>
</tr>
<tr>
<td>7.</td>
<td>Water Insoluble Matter</td>
<td>2.27 ± 0.11</td>
<td>% (by Mass)</td>
</tr>
<tr>
<td>8.</td>
<td>Moisture Content (Loss on drying)</td>
<td>8.31 ± 0.012</td>
<td>% (by Mass)</td>
</tr>
</tbody>
</table>

2.5.1 Preliminary Phytochemical Screening

The qualitative screening of ADPHF6 aqueous extract revealed the presence of phytochemical components and has been summarized in Table 6. The phytochemical analysis of ADPHF6 exhibited appreciable amount of active components such as alkaloids, flavonoids, cardiac glycosides, phenols, tannins, terpenoids and saponins. The other bioactive components involving steroids, reducing sugar and lipids & fat are measured to be in minimal amount. The quantitative estimation of total phenols & flavonoids in ADPHF6 extract was represented in Fig 23. The total phenol content was estimated as 473.3±3.05 mg/g equivalents of gallic acid. The amount of flavonoids was estimated as 664±5.29 mg/g of quercetin equivalents.
Table 6: Qualitative Phytochemical Screening in Polyherbal Formulation ADPHF6

<table>
<thead>
<tr>
<th>S.No</th>
<th>Phytochemical Constituents</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>++</td>
</tr>
<tr>
<td>2.</td>
<td>Flavonoids</td>
<td>++</td>
</tr>
<tr>
<td>3.</td>
<td>Phenols</td>
<td>++</td>
</tr>
<tr>
<td>4.</td>
<td>Tannins</td>
<td>++</td>
</tr>
<tr>
<td>5.</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Terpenoids</td>
<td>++</td>
</tr>
<tr>
<td>7.</td>
<td>Anthrones</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>Saponins</td>
<td>++</td>
</tr>
<tr>
<td>9.</td>
<td>Reducing sugar</td>
<td>+</td>
</tr>
<tr>
<td>10.</td>
<td>Cardiac glycosides</td>
<td>++</td>
</tr>
<tr>
<td>11.</td>
<td>Lipids &amp; Fat</td>
<td>+</td>
</tr>
</tbody>
</table>

- = Absent, + = Present, ++ = Appreciable Amount.
2.5.2 In-vitro antioxidant Assays

Aqueous extract of ADPHF6 possesses significant inhibition for stable DPPH free radical with IC$_{50}$ value of 49.9±0.15 mg/ml, as compared to standard ascorbic acid IC$_{50}$ value of 60.1±0.16 mg/ml (Fig. 24). For Hydroxyl radical scavenging assay, ADPHF6 possesses similar substantial quenching activity with IC$_{50}$ value of 66.1±0.10 mg/ml, compared to standard ascorbic acid IC$_{50}$ value of 84.0±0.11 mg/ml (Fig. 25). The chelating activity of ADPHF6 aqueous extract for ferrous ions Fe$^{2+}$ was measured as IC$_{50}$ value of 76.1±0.01 mg/ml, as compared to standard EDTA 93.4±0.01 mg/ml (Fig. 26).

2.5.3 α-amylase & α-glucosidase inhibition Assay

Aqueous extract of ADPHF6 possesses significant inhibition for α-amylase with IC$_{50}$ value of 0.98±0.01 mg/ml (P < 0.05) (Table 7), compared to Acarbose IC$_{50}$ value of 1.41±0.01 mg/ml (Fig. 27). Similarly, for α-glucosidase inhibition assay, ADPHF6 possess momentous inhibition with IC$_{50}$ value of 0.67±0.01 mg/ml (P < 0.05) (Table 7), as compared to Acarbose IC$_{50}$ value of 0.81±0.01 mg/ml (Fig. 28).
Fig 24: DPPH Radical Scavenging Assay

Fig 25: H$_2$O$_2$ Radical Scavenging Assay
Fig 26: Metal Chelating Activity

Fig 27: α- Amylase Inhibition Assay
**Fig 28: α- Glucosidase Inhibition Assay**

**Table 7:** ANOVA for antioxidant activity (DPPH Radical Scavenging Assay, Hydrogen Peroxide (H₂O₂) Radical Scavenging Activity & Metal chelating activity); Alpha Amylase & Glucosidase inhibitory activity by ADPHF6 aqueous extract.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Df</th>
<th>MS</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPH Radical Scavenging Assay</td>
<td>4</td>
<td>2680</td>
<td>P &lt; 0.0001*</td>
</tr>
<tr>
<td>Hydrogen Peroxide (H₂O₂) Radical Scavenging Activity</td>
<td>4</td>
<td>824.8</td>
<td>P &lt; 0.0001*</td>
</tr>
<tr>
<td>Metal Chelating Activity</td>
<td>4</td>
<td>1946</td>
<td>P &lt; 0.0001*</td>
</tr>
<tr>
<td>α-Amylase Inhibition Assay</td>
<td>5</td>
<td>271.0</td>
<td>P &lt; 0.0001*</td>
</tr>
<tr>
<td>α-Glucosidase Inhibition Assay</td>
<td>5</td>
<td>905.3</td>
<td>P &lt; 0.0001*</td>
</tr>
<tr>
<td>Lipid Peroxidation (Pre &amp; Post) (TBARS)</td>
<td>4</td>
<td>368.6</td>
<td>P &lt; 0.0001*</td>
</tr>
</tbody>
</table>
By Tukeys test (Table 7), Polyherbal Formulation ADPHF6 exhibits Total variation (%) of 97.23 % (DPPH Radical Scavenging Assay), 91.66 % (Hydrogen Peroxide [H₂O₂] Radical Scavenging Activity), 87.21 % (Metal Chelating Activity), 12.78 % α-Amalyse inhibition Assay, 98.14 % α-Glucosidase Assay & 37.70 % (Lipid Peroxidation) as compared with respective standards. Df indicates Degree of Freedom, MS indicates Mean of Squares. * Significant at P < 0.05 or 0.01, respectively.

2.6 Discussion

World health Organization assessed Diabetes Mellitus (DM) as one of the major endocrine disorder with substantial disruption in carbohydrate, fat & protein metabolism. Though numerous factors such as obesity, age, physical inactivity and genetic predisposition are reflected to play a key role in development of T2DM, our decisive objective will end up in management of T2DM to avoid further micro & macro vascular complications (91, 92). Additionally in some cases, the generation of Reactive Oxygen Species (ROS) will often increase during hyperglycaemic condition due to accumulation of excess glucose and fatty acids in adipose tissue (47, 48). ROS molecules in surplus concentration, has been reported to generate oxidative DNA damage and also cause intervening damage to adjacent macromolecules like Nucleic acids, lipids, proteins and various cell membranes (93). The prime physiological function of immune system is amputation of free radicals such as ROS & its mediated effects. Even though cells are equipped with an impressive range of antioxidant enzymes and antioxidant molecules, these agents may not be sufficient enough to normalize the redox status during oxidative stress in postprandial hyperglycemia (PPHG) circumstances.

At present scenario, though various pharmacological based conventional therapeutic approaches are in practice, controlling postprandial hyperglycaemia will be an expected to
turn an utmost response for the management of T2DM and its mediated complications (94). Effective strategy in managing PPHG is to delay the absorption of blood glucose through the inhibition of carbohydrate hydrolysing enzymes such as α-amylase & α-glucosidase (95, 96). Some synthetic inhibitors like acarbose, migitol & voglibose are in clinical use as a therapy for PPHG condition via inhibition of α-amylase & α-glucosidase enzymes. Further follow-up studies confirmed that, these inhibitors are often ended up with various gastrointestinal associated problems like abdominal discomfort, flatulence, meteorism and diarrhoea (97). These major therapeutic complications of synthetic enzyme inhibitors have witnessed a major turnaround towards the practice of non-synthetic drug.

Active components exist in drugs from natural sources expected to be a value added therapeutic strategy for the management of PPHG. Some of the phytochemical constituents such as alkaloids, flavonoids, cardiac glycosides, phenols and saponins are reported for antioxidant activity and also playing a crucial role in inhibiting the carbohydrate hydrolysing enzymes measured to be substantial quantity in our ADPHF6 polyherbal formulation.

In mammalian system, prime aspect for the development of postprandial hyperglycaemia is due to increased breakdown & absorption of complex carbohydrates by the action of glycosidases enzymes (pancreatic α-amylase [EC 3.2.1.1] & α-glucosidase enzyme [EC 3.2.1.20]) into simple sugar molecules such as maltose & glucose. Inhibition of these glycosidase enzymes resulted in prolonged overall carbohydrate digestion period, which ultimately channels up to reduction in the rate of glucose absorption and subsequently blunts the increased post-prandial blood glucose level (97).

Our present study demonstrates that ADPHF6 offers potent inhibitory activity against α-amylase & glucosidase enzymes as compared to standard Acarbose. From our current chapter, it is evident that antioxidant & anti-hyperglycaemic activity is due to abundant
quantity of bioactive constituents exists in ADPHF6 polyherbal formulation. Our test material also tested for organoleptic & physicochemical properties which is prerequisite to validate the quality & stability of herbal drug. In the next chapter ADPHF6 drug will be studied for brief analytical study, to further elucidate the secondary metabolites responsible for anti-hyperglycaemic activity.
2.7 Reference


61. Huang CS, Yin MC, Chiu LC. Antihyperglycemic and antioxidative potential of \textit{Psidium guajava} fruit in streptozotocin induced diabetic rats. Food and Chemical Toxicology. 2011; 49 (9): 2189-95.


