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EXPLORING THE ROLE OF PHYTOCHEMICALS AND ANTIOXIDANTS ON ANTIHYPERGLYCEMIC POTENTIALS OF INDIAN MEDICINAL PLANTS

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ABSTRACT

Various plants have antihyperglycemic potential, and the present study is emphasised to determine the role of phytochemicals and antioxidants against diabetes, as preventive health care management. The aim of this current research work was sought to analyse aqueous and methanolic extracts of *Momordica charantia*, *Trigonella foenum-graneum*, *Triticum aestivum*, *Solanum tuberosum* and *Nicotiana tobaccum*.The phytochemical screening of aqueous and methanolic extracts of test plants was done to check the presence of charantin, alkaloids, flavonoids, total phenols and tannins, through various *in vitro* assays. The antioxidant capability of extracts is to quench free radical, which was explored spectrophotometrically against reducing, power, nitric oxide, total carotenoids and superoxide anion. The study indicated a significant antioxidant activity and reasonable secondary metabolites, thus supporting its role as an antihyperglycemic component among traditional medicinal practices. Methanolic extracts of bitter melon and fenugreek have a higher amount of charantin (active principle as anti hypoglycemic agent) than its aqueous extract. Other phytochemicals and antioxidant activity were higher in aqueous extract than methanolic extract. The aqueous extract of test plants contained several bioactive phytochemicals that possessed medicinal properties, such as antioxidant activities and antihyperglycemic activities. The active ingredient, if subjected to further purification may further serve as an alternative drug of choice against hyperglycemia.

KEYWORDS: Plants Active Metabolites, Phytochemicals, Antioxidants, Antihyperglycemic Activities

INTRODUCTION

Globally, the incidence of diabetes was estimated to be 171 million, i.e.; 2.8% in 2000 and it may increase up to 366 million i.e.; 4.4% by 2030. Diabetes mellitus is a heterogeneous metabolic disorder, recognised by hyperglycemia, glycosuria, and negative nitrogen balance resulting in various complications as diabeticretinopathy, diabetic-nephropathy, diabeticneuropathy, peripheral vascular disease, atherosclerosis, coronary artery disease, high cholesterol and high blood pressure, etc. Diabetes Mellitus is recognised to man ever since ages, **(Korczowski, 1985)** and numerous treatments for the disease explore from the ancient times, were based on the usage of plants and its parts.**Grover et al., 2002** suggested that old medical systems, like Ayurveda, make use of plants and herbs to combat diabetes. Diets of green vegetables, infusions, decoctions, steeped leaves or stems or simply water extracts have been used since ages **(Ajabnoor and Tilmisany, 1988).**

The free fatty acids, (FFAs) play an important role in the development of insulin resistance (IR), FFAs released from intra-abdominal adipose tissue enters into circulation, and these are involved in various organs (liver, muscles, etc.) of developing symptoms of IR. The excess of FFAs in the liver, leads to stimulation of gluconeogenesis and hepatic glucose

output, which contribute to the development of, increased fasting plasma glucose, and the type 2 diabetes **(Grundy et al. 2005).** Defective insulin secretion or β-cell dysfunction, and insulin resistance contribute to, more or less jointly for the development of the pathophysiological condition. The effects of diabetes mellitus include, long-term damage, dysfunction and failure of various organs. Hyperglycemia alone, does not cause diabetic complications. It is rather the detrimental effect of glucose toxicity, due to chronic hyperglycemia, which is mediated and complicated through oxidative stress. Hyperglycemia-induced activation of, protein kinase-C (PK-C) isoforms **(Klein 1995)**, increased formation of, glucose derived advanced glycationendproducts (AGEs) **(Koya and King 1998)**. And increased glucose flux through, aldose reductase pathways, activation of hexosamine pathway and the formation of Glucosamine are some of the known biochemical mechanisms of hyperglycemia-induced tissue/organ damage **(Brownlee 1995).** Therefore the plants possessing active metabolites, can be used as an alternative therapy to enhance insulin sensitivity, of the cell surface receptors. Apart from currently available therapy, herbal medicines are recommended for treatment of diabetes throughout the world. Herbal drugs are prescribed widely, because of their effectiveness, fewer side effects and relatively low cost **(Venkatesh et al., 2003).** Numerous studies have confirmed the strong association, between a diet rich in plant foods and the healthy positive effects of these foods, whch may rely on their content on phytochemicals, antioxidant, vitamins and fibre. Phytochemicals are bioactive compounds, contained in the plants that have the potential for offering protection against a range of non-communicable diseases like diabetes, cancer, cardiovascular disease and cataract. Currently, it is known that a huge amount of phytochemicals, which mainly consists of flavonoids, glucosinolates, phenolic acids, phytates, phytoestrogens, fats and oils contained in, vegetables, fruits, cereals, legumes and other plant sources **(Surh, 2002).** Polyphenols constitute the most abundant phytochemicals, provided by the food of plant origin, been widely distributed in fruits, vegetables, whole cereals, coffee, cacao, and tea. Polyphenols may have, anti-obesity, anti-inflammatory, anti-diabetic, and anticancer properties through multiple mechanisms: they act by, modulating inflammation and redox state, regulatig adipocyte differentiation and lipid metabolism, inhibiting pancreatic lipase activity and intestinal permeability, and by interacting with the gut microbiota **(Chattopadhyaya et al., 1996).**

According to their chemical structure, polyphenols were classified into different categories: phenolic acids, stilbenes, flavonoids, chalcones, lignansand and curcuminoids. Ferulic acid, a phenolic acid present in whole wheat, has been proved to be effective against, high fatinduced hyperlipidemia and oxidative stress, via regulation of insulin secretion and, regulation of antioxidant and lipogenic enzyme activities **(Lacueva et al., 2011).** Alkaloids, primarily found in red hot peppers and sweet peppers, exert pharmacological and physiological actions, including anticancer, anti-inflammatory, antioxidant, and anti-obesity effects, **(Luo et al., 2011). Tan et al. (2008)** demonstrated that, an extract from bitter melon (*Momordicacharantia*) had agonist activity of AMP activated protein kinase, (AMPK) in 3T3-L1 adipocytes and enhanced glucose disposal in, insulin-resistant mice. In a retrospective sub-analysis, of participants with higher cardiovascular disease risk. **Lerman *et al.* 2010** found that, the additional phytochemical supplementation needed to adequately reduce multiple biomarkers, associated with cardiovascular disease in this high-risk population. Pharrmacological interference of these targets with anti-diabetic agents have undesirable side effects. Due to the richness and complexity of the compounds in plants, herbal therapy has always been thought to act on multiple targets in the human body. Even one single compound can have multiple targets, the multiple targets associated with anti diabetic herbal medicine make clinical trials complicated, but such an approach is more likely to lead to an eventual cure.

The present study deals with, the phytochemical profiling of the seeds and leaves of, five test plants possessing antihyperglycemic activity.

Since the chemicals/medicines used do possess some side effect for uptake for a long duration, that gives us the impetus to analyse and screen the plants, and evaluate the nature of the active ingredient, responsible for their potential activity.

MATERIALS AND METHODS

Test Plants

Five plants were used for the analysis of their antihyperglycemic activity in their seeds and leaves, like bitter melon (*Momordicacharantia*), fenugreek (*Trigonellafoenum-graecum*), wheat (*Triticumaestivum*), potato (*Solanumtuberosum*), and tobacco (*Nicotianatobaccum*). Seeds were collected from the local market and were sown in earthern pots, thereby providing optimal nutrients, an adequate amoun of light and water fo its growh. the harvested leaves were used fo study conduct.

Plant extract was prepared by soaking, 2grams of ground seeds in 0.5 ml of, distilled water (aqueous extract) and in methanol (methanolic extract) for overnight. The ground seeds were subjected to aqueous as well as methanolic extraction, for the isolation and quantitation of various phytochemicals, respectively. Similarly, extracts from the leaves were also prepared for the study conduct.

Phytochemical Screening of, Various Secondary Metabolites/Antioxidants in the Test Plant's

Charantin (Steroidal Saponin)

Plant material was pulverised into fine powder. 100gm of samples were soaked in water/ethanol solvent, from 1-72 hour under, temperature 30-80◦C. pH of the mixture, and was adjusted from 2-13 with buffer solutions at pH 3-6, which is 10mM citrate phosphate; pH 7 is 10mM sodium phosphate; pH 8 is 10mM Tris-HCl; pH 9-10 is 10mM carbonate - sodium bicarbonate; and pH 11-12 is 10mM glycine-NaOH. The extract was cooled in a water bath, and was collected in fractions in sample collecting vials, every 4 hours in the1st day. Then collected every 6 hours in the 2nd and 3rd day. Extracts were evaporated under vacuum, to remove water-ethanol solvent. Mixtures run through th Si gel column to separate, charantin. Further identified on HPLC, using reference standards and for protein, Sephadex (G20 and G50) was used **(Mohamad et al., 2011).**

Alkaloids

5g of the sample was taken with 250 ml of, 20% acetic acid in ethanol and kept for 4hrs. Extract filtered, and the extract was concentrated using the water bath, until the volume reduces to one-fourth of the original volume. Then, concentrated NH_4OH was added dropwise, to the extract until the precipitation was complete. The whole solution was allowed to settle, and the precipitate was collected by filtration and weighed **(Harborne, 1973; Obadoni and Ochuko, 2001).**

Saponins

Grind the plant sample, and take 20 grams of each plant sample. Add 100ml of 20% ethanol in it and shake for 30 minutes. Heat on a water bath at 55°C for 4 hours, and filter it and concentrate the filtrate till 40%, on a water bath. Add 20ml diethyl ether and discard the upper layer of it. Take lower (aqueous) layer and add 60ml n-butanol in it. Add 10ml of 5% NaCl in it. Heat till dry (oven dry/40°C) and weigh it **(Surendar et al. 2011).**

Flavanoids

The antioxidative properties of flavonoids are, due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, and inhibition of enzymes, responsible for free radical generation **(Benavente-Garcia, 1997).** Depending on their structure, flavonoids can scavenge practically all known ROS. The amount of total flavonoid content, can be determined by Aluminium chloride method **(Chang et al., 2002).** The reaction mixture, (3.0 ml) comprised of 1.0 ml of extract, 0.5 ml of aluminium chloride (1.2%) and 0.5 ml of potassium acetate (120 mM) was incubated at room temperature for 30 mi,n and absorbance measured at 415 nm. Quercetin **(Ordonez et al., 2006)** or catechin (Kim et al., 2003) can be used as a positive control. The flavonoid content was expressed with respect to the standard equivalent (mgg-1 of extracted compound).

Total Phenolic Content

Plant polyphenols, a diverse group of phenolic compounds (flavanols, flavonols, anthocyanins, phenolic acids, etc.), possess an ideal structural chemistry for free radical scavenging activity. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors from the ability of the polyphenol derived radical to stabilise and delocalize the unpaired electron (chain-breaking function) and from their potential to chelate metal ions (termination of the Fenton reaction) **(Rice-Evans et al., 1997).**The amount of total phenol content can be determined by Folin-Ciocateu reagent method **(McDonald et al., 2001).** 0.5 ml of extract and 0.1 ml of Folin-Ciocalteu reagent (0.5 N) are mixed and incubated at room temperature for 15 min. Then 2.5 ml saturated sodium carbonate is added and further incubated for 30 min at room temperature and absorbance measured at 760 nm. Gallic acid **(McDonald et al., 2001),** tannic acid **(Wolfe et al., 2003),** quercetin **(Singleton and Rossi, 1965),** chlorogenic acid **(Singleton et al., 1999),** pyrocatechol **(Slinkard and Singleton, 1977)** or guaiacol **(Yildrim et al., 2001)** can be used as positive controls. The total phenolic content was expressed on the standard equivalent (mgg-1 of extracted compound).

Tannins

500mg of the sample was weight into the100ml plastic bottle, 50ml of distilled water was added and shaken for 1h in a mechanical shaker. The reaction mix was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filtrate was pipette out into a tube and mixed with 3ml of 0.1M FeCl3 in 0.1N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured with a spectrophotometer at 120nm wavelength, within 10mins. A blank sample without plant extracts was prepared, and absorbance recorded at the same wavelength. A standard was prepared using tannic acid to get 100 ppm and measured the absorbance **(Imran et al. 2012).**

Reducing Power

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity (Oktay et al., 2003). Compounds with reducing power indicate that they are electron donors and can reduce the oxidised intermediates of lipid peroxidation processes so that they can act as primary and secondary antioxidants **(Yen and Chen, 1995).** The reducing power can be determined by the method of **Athukorala et al. (2006).** 1.0 ml extract is mixed with 2.5 ml of phosphate buffer (200 MM, pH 6.6) and 2.5 ml of potassium ferricyanide (30 MM) and incubated at 50°C for 20 min. After that, 2.5 ml of trichloroacetic acid (600 MM) is added to the reaction mixture, centrifuged for 10 min at 3000 rpm.

The upper layer of solution (2.5 ml) is mixed with 2.5 ml of distilled water, and 0.5 ml of FeCl3 (6 MM) and absorbance measured at 700 nm. Ascorbic acid, butylatedhydroxyanisole (BHA), tocopherol, trolox (Oyaizu, 1986) or butylatedhydroxytoluene (BHT) **(Jayaprakasha et al., 2001)**was used as positive control.

Nitric Oxide

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interact with oxygen to produce nitrite ions, which were measured using the Griess reaction reagent **(Green et al., 1982).** 3.0 ml of 10 MM sodium nitroprusside in phosphate buffer was added to 2.0 ml of extract and reference compounds in different concentrations (20 - 100 _g/ml). The resulting solutions are then incubated at 25°C for 60 min. A similar procedure is repeated with methanol as blank, which serves as a control. To 5.0 ml of the incubated sample, 5.0 ml of Griess reagent (1% sulphanilamide, 0.1% naphthyethyl-one-diaminedihydrochloride in 2% H3PO3) was added, and the absorbance of the chromophore formed is measured at 540 nm. Percent inhibition of the nitrite oxide generated was measured by comparing the absorbance values of control and test preparations. Curcumin, caffeic acid, sodium nitrite **(Rao, 1997),** BHA, ascorbic acid, rutin **(Jayaprakasha et al., 2004),** BHT or tocopherol **(Garrat, 1964)**used as a positive control.

Superoxide Activity

The ability of the WEN to scavenge hydrogen peroxide was determined according to the method of **Ruch et al. (1989).** A solution of hydrogen peroxide (40 MM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm in a spectrophotometer (8500 II, BioCrom GmbH, Zurich, Switzerland). Extracts (50–250 g) in distilled water was added to a hydrogen peroxide solution (0.6 ml, 40 MM). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing in phosphate buffer without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of WEN and standard compounds was calculated using the following equation:

Percent Scavenged [H_2O_2] = (A0 − A1) /A0 × 100

Where A0 was the absorbance of the control, and A1 was the absorbance in the presence of the sample of WEN and standards **Gülçinet al. 2003.**

Total Carotenoids

Samples were prepared according to **Lachman et al. (2003).**The absorbance of methanolic and aqueous extracts was then measured in 1 cm cuvettes at λ = 444 nm against acetone (SpectronicHeλios γ spectrophotometer, Thermo Electron Corporation, GB) and total carotenoid content in mg/kg fw of the sample was expressed as the lutein equivalent from the equation:

$(K + X)L = (A_{444*}\ 25\ *\ 15)/0.259*m$ (mg/gm fw)

Where: $(K + X)$ L is total carotenoid content (carotenes and xanthophylls) $A444$ is absorbance of acetone extract at λ = 444 nm m is a sample weight (g) **Kotíkova et al. 2007.**

RESULTS AND DISCUSSIONS

Hyperglycemia, abnormal lipid metabolism and antioxidant profiles are the most usual complications in diabetes mellitus. Since to treat diabetes and related complications, a drug possessing antidiabetic, lipid-lowering and antioxidant

activities altogether are supposed to be more effective. Use of antioxidants and phytochemicals can be a great help in tissue repair by quenching the free radicals generated due to oxidative stress. If the mechanism for stress generated hyperglycemia revealed, the target driven therapeutic approach can be performed involving phytochemicals. More than 300 Indian medicinal plants are known to be anantidiabetic but the exact mechanism for the hypoglycemic action of only a few is known. Many herbal medicines as single agents or in different oral formulations can be recommended for diabetes mellitus because they have lesser repercussions than oral hypoglycemic agents such as sulfonylurea, metformin and triglitazone etc.

During the study, phytochemical screening of various secondary metabolites present in the test plants (seeds) in the aqueous and methanolic fraction had been done. It has been summarized that from the seedsmaximum amount of alkaloids was recovered from wheat in aqueous fraction and bitter melon in amethanolic fraction. The maximum amount of flavanoids recovered from tobacco in an aqueous and methanolic fraction. The maximum amount of saponin was recovered from fenugreek in an anaqueous and methanolic fraction. The maximum amount of tannins was recovered from potato in aqueous fraction and bitter melon in amethanolic fraction. The maximum amount of total phenol was recovered from bitter melon in aqueous fraction and fenugreek in amethanolic fraction. Similarly, momordicoside(s) enhanced fatty acid oxidation and glucose disposal during glucose tolerance tests in both insulin-sensitive and insulin-resistant mice. These findings indicate that cucurbitanetriterpenoids, the characteristic constituents of *M. charantia*, may provide leads as a class of therapeutics for diabetes and obesity Tan *et al.* 2008.The comparative study also indicated that the hypoglycemic effect of ethanol extract of fenugreek seeds and that of isolated pure trigonelline was not significant ($P > 0.05$). In conclusion, based on the result of this study, trigonelline at a dose of 10mg/12hrs has a significant hypoglycemic effect in both normal and diabetic rabbits. It is preferred to use trigonelline to avoid the possible adverse reactions which may appear due to the multi-components of the ethanol extract of the fenugreek seeds **Al-Khateeb *et al.* 2012.**The extract of fenugreek seeds contains antioxidants and protects cellular structures from oxidative damage. These findings provide evidence for the in vivo beneficial effects of the seeds reported in the literature **Kaviarasan *et al.* 2007.**The antioxidant activities of flesh, inner tissue, and seed ranged from 81.7% to 86.5%, 78.8% to 88.4%, and 78.5% to 85.4% inhibition, respectively. Bitter melon is a rich source of phenolic compounds. These natural plant phenolics can be a good source of antioxidants for application in food system **Horax*et al.* 2005.**A synthetic wheat experimental line, W7985, gave the lowest carotenoid concentrations of any of the genotypes in this study. Such large genotypic differences in carotenoid content may open up new opportunities for breeding wheat varieties with higher nutritional value **Adom *et al.* 2003.**

Antioxidant profile of test plants from seeds has been done, and it has been observed that maximum activity of reducing power was recovered from fenugreek in an aqueous and methanolic fraction. The maximum activity of nitric oxide was recovered from potato in aqueous fraction and wheat in amethanolic fraction. The maximum amount of total carotenoids was recovered from tobacco in an aqueous and methanolic fraction. The maximum activity of H_2O_2 scavenging was recovered from potato in aqueous fraction and wheat in amethanolic fraction.

CONCLUSIONS

Since, plants possess active metabolites that act against hyperglycaemia, in the future it can be used as a potent alternative to the medication. If these plants will be used after optimization along with the medication can reduce the total dosage daily consumption and thereby reducing the severity of the adverse effects of long term usage of the medicines. Recently, it has been decided by Indian government “Pioglitazone”, an antidiabetic drug was sold with certain conditions

as it causes bladder cancer (based on the report in an article in the Times of India dated 06.08.2013). Whereas antihyperglycemic components isolated from test plants are more effective than allopathic treatment as they have no side effects, it can be recommended to be used alone or in combination with the chemical formulations. Such reports give us the impetus to explore the analysis of the mechanism of action of these components under *in vivo* i.e.; in animal cell lines to observe the bioefficacy of these components in the animal model system. We can further analyze the impact of these plant preparations under *in vivo* system for the dosage optimization.

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Compliance with Ethics Guidelines

There is no conflict of interest and there are no ethical issues with human or animal subjects in the current study.

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APPENDICES

Table 1: Quantity of the Alkaloid, Flavanoid, Saponinn, Tannins, Total Phenol Content from Seeds of the Test Plants in Anaqueous and Methanolic Fraction

SNO.	Plant Sample	Alkaloid (gm/gm)	Flavanoids (gm/gm)	Saponin (gm/gm)	Tannins (mg/ml)	Total Phenolic content (mg/ml)
A.	**Aqueous fraction**					
1)	Bittermelon	0.069	0.029	0.044	0.061	0.791
2)	Fenugreek	0.069	0.027	1.013	0.080	0.290
3)	Wheat	0.086	0.027	0.047	0.119	0.209
4)	Potato	0.072	0.035	0.037	0.986	0.067
5)	Tobacco	0.065	0.229	0.026	0.042	0.551
B.	**Methanolic fraction**					
1)	Bittermelon	0.103	0.009	0.012	0.156	0.213
2)	Fenugreek	0.069	0.037	0.041	0.091	0.352
3)	Wheat	0.081	0.06	0.011	0.076	0.112
4)	Potato	0.073	0.042	0.026	0.066	0.071
5)	Tobacco	0.087	0.055	0.016	0.077	0.349

Table 2: Antioxidant Profile of the Test Plants from Seeds in Anaqueous and Methanolic Fraction

	Bittermelon		Fenugreek		Wheat		Potato		Tobacco	
	Aq	Meth	Aq	Meth	Aq	Meth	Aq	Meth	Aq	Meth
Reducing Power (mg/gm)	0.843	0.89	1.3872	1.337	1.251	0.568	0.393	1.178	1.087	0.535
Nitric Acid (mg/gm)	1.058	2.744	3.315	1.65	3.095	3.549	3.618	2.306	2.889	2.8
Total Carotenoid (kg/kg of w)	26.053	5.096	14.884	5.241	2.533	0.622	2.316	0.086	108.503	11.206
H_2O_2 Scavenging Activity(%)	-12.07	-2.84	12.976	-25.8	-4.02	18.205	31.439	4.067	-15.364	13.621

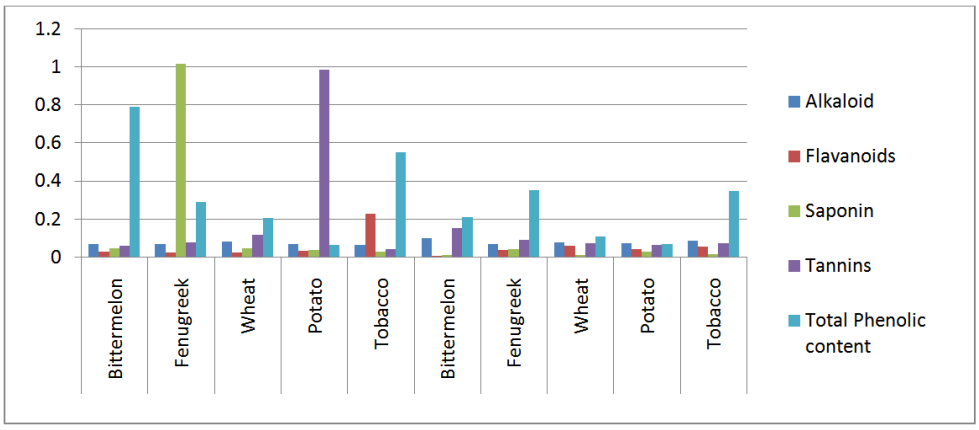

Figure 1: Histogram Showing the Quantity of Alkaloid, Flavanoid, Saponin and Total Phenolic Content of Seeds of Test Plants in Anaqueous and Methanolic Fraction