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Comparative Study on Anti Diabetic Property of *Syzyium cumini, Aegle marmelos and Cocos nucifera* through *in vitro* and *in vivo* Condition

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Abstract: ***The aim of the present study was to investigate presence of antidiabetic activity in plant extracts like Syzyium cumini, Aegle marmelos and Cocos nucifera using a solvent like methanol and aqueous. In preliminary phytochemical analysis and Paper Chromotography, different types of phytocompounds like alkaloids, flavonoids, phenols, glycosides and saponins were present, which it had been concluded that there is rich in phyto compounds for the antidiabetic activity which is highly responsible for regulating the pancreatic hormone for the synthesis of insulin. In the in vitro antidiabetic analysis, among all the three plant extracts, aqueous extract of Cocos nucifera have high sugar reducing capacity. Aqueous extract of Cocos nucifera were taken for HPLC analysis and antioxidant activity. HPLC analysis of Cocos nucifera resulted in attaining a sharp peak with retention time of 1.983. It was closely related to stevioside compounds. Analysis of antioxidant activity in Cocos nucifera shows 69.8% of reductions in DPPH free radicals for 100µg/ml. Aqueous extract of Cocos nucifera were further taken for in vivo study. The mice were made diabetic by intraperitoneal administration of 150mg/kg of alloxan. Finally, blood sugar reduces from 408mg/dl to 88 mg/dl when treated with endocarpic extract of coconut.***

Keywords: Cocos nucifera, antidiabetic, HPLC, alloxan, Intraperitoneal

1. Introduction

Diabetes mellitus (DM) is a complex and a diverse group of disorders that disturbs the metabolism of carbohydrate, fat and protein. The number of diabetes mellitus cases has been increasing worldwide in recent years. In 2000, the World Health Organization estimated a total of 171 million of people with diabetes mellitus from the global population, and this report projected to increase to 366 million by 2030. Diabetes is becoming the third killer of mankind, after cancer and cardiovascular disease, because of its high prevalence, morbidity and mortality. The number of adults suffering from diabetes in India is expected to increase three fold, from 19.4 million in 1995 and 57.2 million in 2025[1].

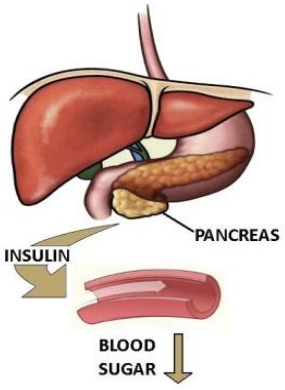

Figure 1: Role of insulin

Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic diseases in which there are high blood sugarlevels over a prolonged period. Insulin is an only growth harmone that could regulate the blood sugar level in the body as mentioned by fig: 1.

At present the treatment of diabetes mellitus is based on oral hypoglycemic agent and insulin. An almost artificially synthesized drug brings out some side effects. Human beings have to depend on nature since his existence for survival. Using his knowledge man has discovered many remedies for ailments from nature such as plants, mineral materials and animal products [2]. The history of drug is intimately linked with plants from the earliest times and even today plant products have extensive use in ethno medicine, traditional systems of medicines as well as in the armamentarium of the modern physician. The interest in the study of medicinal plants as a source of pharmacologically active compounds has increased worldwide. It is recognized that in developing countries like India, plants are the main medicinal source to treat infectious diseases [3].

Diabetes mellitus (DM) is also treated by Indian traditional medicine using anti-diabetic medicinal plants. However, herbs are not inexhaustible natural resources and the demand for herbal medicines can't be met by cultivation. With a long course and serious complications often resulting in high death-rate, the treatment of diabetes spent vast amount of resources including medicines, dietary guidelines, physical training and so on in all countries. Thus searching for a new class of compounds is essential to overcome diabetic problems. There is continuous search for alternative drugs because the existing synthetic drugs have several limitations. Many oral hypoglycemic agents like Sulphonylurea, liguanides, thiaolidivediones, meglitinide derivatives and α - glucosidase inhibitors are presently in use but they all have several side effects. The herbal drugs with anti diabetic activity are yet to be commercially formulated as modern medicines, even though they have been acclaimed for their therapeutic properties in the traditional systems of medicine. [4]

The endocrine pancreas consists of approximately 1 million islets of Langerhans interspersed throughout the pancreatic gland. Within the islets, at least four hormone-producing cells are present. Their hormone products include insulin, islet amyloid polypeptide (IAPP, or amylin), whose metabolic function remains undefined glucagon, the hyperglycemic factor that mobilizes glycogen stores; somatostatin, a universal inhibitor of secretory cells, and pancreatic peptide enzyme, a small protein that facilitates digestive processes by a mechanism not yet clarified.

The ethanobotanical information reports about 800 plants that may possess anti diabetic potential. *Syzyium cumini* (or) *Eugenia Jambolona* tree belongs to the Myrtaceae family. This is also called as Jamun and Jambul in India and Malaya. It is one of the most commonly medicinal plants used to treat diabetes mellitus in Brazil. Different parts of this plant, such as seeds, bark, fruit and leaves have been used in traditional medicine as a remedy for diabetes mellitus in many country. The seeds are sweet, astringent to bowels and good for diabetes [8]

Aegle marmelos (L) Core, (Rutaceae) is a popular medicinal plant in the Ayurvedic and siddha systems of medicines used to treat a wide variety of ailments. The plant, popularly known as the bale tree, is native to the Indo-Malayan region and is currently cultivated in India, Pakistan, Bangladesh, Srilanka, Burma and Thailand [9].The folkloric use of this species to treat infectious diseases stimulated in human body. It is a large ever green tree upto 30m height, the leaves measuring about 10 to 15 cm long and 4 to 6 cm wide. The leaf of *Aegle marmelos* is used for ophthalmic, diabetes and asthmatic complaints. It enhances the ability to utilize the external glucose load in the body by stimulation of glucose uptake similar to insulin [10].

Cocos nucifera is an important member of the family Araceae (palin family) and it is the only accepted species of the genus cocos. The English name is coconut. It has a long history of use in traditional medicine for the treatment of metabolic disorders. The most interesting feature of the fruit is its wall. The fruit wall comprises of three layers exocarp, mesocarp and endocarp. Endocarpic extract of tender coconut act as antidiabetic agent [5]

By chromatographic methods coupled to mass spectrometry techniques, it has been demonstrated that aqueous *Cocos nucifera* extracts are mainly composed of catechinand alkaloids. These classes of molecules have been associated with analgesic, antidiabetic and antioxidant in several experimental models. The present study compares the methanol and aqueous of the three plants extracts by their phytochemical (Tube test, TLC and HPLC), anti oxidant, anti bacterial and anti diabetic property through *In vitro* condition and the plant with best glucose degrader were taken for *in vivo* condition. [7]

2. Materials and Methods

2.1.1. Collection of plant material

The leaves of *Aegle marmelos, Syzium cumini* and *Cocos nucifera* were collected from sadhuragiri hills at Western Ghats. The plant parts were washed with distilled water. Washed plant leaves of bael and jambul were air dried in darkness at room temperature for 4 days. Endocarpic tender coconut was freshly taken without dryness. Dried and undried plant parts were powdered using a mechanical mixer. [8]

2.1.2. Plant extraction:

10gm powdered leaves of *Aegle marmelos, Syzyium cumini* and endocarpic membrane of tender coconut were extracted by using macerate technique. [6]

2.2 Identification of the Plant Constituents by Phytochemical Test:

Small quantity of aqueous and methanolic extracts of *Aegle marmelos, Syzyium cumini* and *Cocos nucifera* were taken and are used for detection of phytochemical study.[5]

2.3 Analysis of phyto compounds in paper chromatography:

Phyto compounds were analysed in paper chromatography, Few drops of aqueous and methanolic extract of the three plant extracts were spotted on the line of paper with the help of capillary tube. The paper was placed the developing jar with mobile phase.

The RF values were calculated by the formula,
Retention factor (RF) =
Distance travelled by solvent from origin
Distance travelled by solute from origin

2.4 Phyto chemical analysis of *Cocos nucifera* extract by HPLC (High Performance Liquid Chromotography)

HPLC of crude aqueous extract of *Cocos nucifera* plant was carried out by SHIMADZU, LC-10AT VP (25 X 0.5cm 10A) columns. Mobile phase used was Acetonitrile and Phosphate Buffer (35:65). 20 µl of the volume was injected with the flow rate of 1ml/min. Detection wavelength was 268 nm and the method was carried out at ambient temperature. Isocratic method was used for obtaining chromatogram of metabolites of *Cocos nucifera.*

2.5 Determination of anti-oxidant activity

DPPH radical scavenging activity

Aqueous extract of *Cocos nucifera* was used to assess the *in vitro* antioxidant activity. Antioxidant scavenging activity was studied using 1,1-diphenyl, 2-picryl hydroxyl free radical (DPPH). The DPPH free radical scavenging activity was calculated using the following formula:
% scavenging= [Absorbance of control – Absorbance of test sample/Absorbance of control] ×100

2.6 Anti diabetic activity:(*in vitro*)

2.6.1. Preparation of test solution

Glucose stock was prepared in the concentration of 1mg/ml. 5ml and 10ml of extracts were taken for sugar degradation. 2ml of glucose stock solution was added to all the extract

concentrations. These tests were done in triplicates. The test solutions were incubated at room temperature for five days.

2.6.2 Preparation of anthrone reagent

200mg of anthrone powder were dissolved in 5ml ethanol and are added with 75ml of ice cold 95% H_2SO_4.

2.6.3. Spectrophotometric analysis

1ml of all the test sample was taken along with addition of 5ml anthrone reagent. The mixtures were kept for incubation in water bath for 10min at 45-50^{0}C. Then 3ml of mixture solution were taken in the cuvette tubes for absorbency reading at 625nm, against blank solution (1ml of distilled water with 5ml anthrone reagent).

Calculation

Concentration of sugar of unknown sample = Concentration of known sample x Optical density of unknown sample/ Optical density of known sample.

2.6.4. Anti diabetic activity: (*in vivo*)

Oral glucose tolerance test:

On the first day of the experiment, a group of four mice were subjected to oral glucose tolerance test (OGTT). The glucose levels were measured using a complete blood glucose monitoring system. Then the results were observed.

2.6.5. Intraperitoneal hypoglycemic activity

Diabetes was induced by injecting alloxan (150 mg/kg body weight). Intraperitoneal treatment done in all the mice according to the following procedure.

Experimental design: The mice were grouped into 4 groups, comprising of 4 mice in each group as follows:

Group I: Control mice

Normal mice with no diabetic inducers.

Group II: Alloxan induced diabetic mice.

Mice without treated by standard and plant extract as drugs. These are assumed as negative control.

Group III: Diabetic mice treated with *Cocos nucifera* endocarpic extract (300 mg/Kg Body weight/day) in aqueous solution intravenously for 7 days.

Group IV: Diabetic mice treated with glibenclamide (5mg/Kg body weight/day) in aqueous solution intravenously for 7 days.

During the experimental period, body weight and blood glucose levels of all the mice were determined at regular intervals.

3. Results and Discussion

Plants have been used for the treatment of diseases throughout the world since in the beginning of civilization. It was popularly called as Ayurvedic and Unani system of medicine. The nutritional and pharmacological properties of the whole herb in its natural form as it has been traditionally used and may results from synergistic interactions of many different active phytochemicals.

Pharmacological activity and different parameters of plant extracts have been resulted as follows,

Table 1: Results of preliminary phytochemical tests

S.no	*Phytocompounds*	*Syzyium cumini*		*Aegle marmelos*		*Cocos nucifera*	
1.	Alkaloids	+	+	+	+	+	+
2.	Carbohydrates	-	-	-	-	-	-
3.	Saponins	+	+	+	+	+	+
4.	Phytosterols	+	+	+	+	+	+
5.	Phenols	-	+	+	+	+	+
6.	Tannins	-	-	+	+	+	+
7.	Flavanoids	-	+	+	+	+	+
8.	Diterpenes	+	+	+	+	+	+
9.	Steroid	-	-	-	-	+	-
10.	Terpenoid	-	-	+	+	-	+
11.	Pholabtannin	-	-	-	-	+	-
12.	Cycloglycosides	+	+	-	+	+	+
13.	Quinone	-	+	+	+	+	+
14.	Glycosides	-	-	-	-	-	+
15.	Anthroquinone	-	-	-	-	-	-
16.	Leucoanthocyanin	-	-	-+	-	-	-
17.	Fatty acids	+	+	+	+	+	+
18.	Cardiac glycosides	-	-	+	+	+	+

3.1 Preliminary phytochemical analysis

The preliminary phytochemical screening tests for methanol and aqueous extracts were done with standard reagents mixture. The presence and absence of phyto compounds were assumed as results and are tabulated in table, 1.

3.2 Paper chromotography

Using standard formula Rf values were calculated and the compounds for respectable Rf values were tabulated in table 2,

Table 2: Results for thin layer chromotography

S.No	*Plant extracts*	*Retention factor [Rf value]*
1.	*S.cumini* (water)	0.84
2.	*S.cumini* (methanol)	0.50
3.	*A.marmelos* (water)	0.66
4.	*A.marmelos* (methanol)	0.88
5.	*C.nucifera* (water)	0.38
6.	*C.nucifera* (methanol)	0.46

Rf value of the three plant extract is closely related to the following compounds,

0.66=Terpenoid, 0.88= caffeine (alkaloid)

0.84= closely related to Flavanoids, 0.50=Papaverine (alkaloid)

0.38= closely related to Protopine and Strychnine (alkaloid), 0.46 = Terpenoid

3.3 HPLC analysis of *Cocos nucifera* extract:

The HPLC chromatograms for an aqueous extract of *Cocos nucifera* is shown in table 3,

Table 3: Results for HPLC test

S.no.	*Area [%]*	*Height [%]*	*WO5 [min]*	*Retention Time[min]*	*Phyto compound*

1.	30.3	52.0	0.13	1.983	Stevioside
2.	63.9	43.2	0.19	2.133	Solanesol [terpenoid]
3.	5.2	3.7	0.19	3.063	Indole Alkaloids
4.	0.6	1.1	0.11	6.297	Quercetin [phenols]

3.4 Anti oxidant activity

The result for analysis of antioxidant activity in *Cocos nucifera* were tabulated in table:7,

Table 7: Results for *in vitro* antidiabetic activity

S.no	*Concentration (mg/ml)*	*Scavenging of DPPH(%) -C.nucifera (water)*	*Results in Percentage*
1.	DPPH Control	0.762	-
2.	5	0.317	51.44%
3.	10	0.306	59.81%
4.	20	0.302	60.33%
5.	40	0.301	60.49%
6.	60	0.250	67.19%
7.	80	0.246	68.50%
8.	100	0.231	69.81%

3.5 Antidiabetic activity (*in vitro*)

All the concentration of the plant extracts contain sugar degrading capacity. Among these, aqueous extract of *Cocos nucifera* reduces sugar up to 76mg/ml in day V and the results were tabulated in table 7,

3.6 *in vivo* anti diabetic activity

Results for *in vivo* antidiabetic activity in oral glucose tolerance test were tabulated in table 8,

Table 8: Oral glucose tolerance test

Animal body weight	*Drug and treatment*	*Normal blood glucose level (before) mg/dl*	*Blood glucose level after drug administration (in min) mg/dl.*		
			30	60	90
H-140 B-120 T-120 C-130	Control Tween 80 + Glucose 2g/kg p.o	088	121	141	148
H-100 B-130 T-120 C-110	Glibenclamide 600µg/kg p.o + Glucose 2gm/kg p.o	089	090	092	088
H-120 B-100 T-110 C-120	Sample 200 mg/kg p.o	088	091	095	094

Figure 2: Oral glucose tolerance test

Results for comparison of natural and chemical drugs was tabulated in table 9,

Table 9: Comparison of allopathy drug and *Cocos nucifera* extract in *in vivo* condition

G. No	*Animal body weight (gm)*	*Drug and treatment*	*Blood glucose level*		
			Before injecting alloxan	*After 48 hrs of alloxan induction*	*After drug administration 0 – 7 days*
I	H-140 B-130 T-140 C-110	Control	74 75 76 72	75 78 82 89	65 64 64 77
II	H-140 B-120 T-150 C-110	Alloxan 150 mg/kg i.p	78 77 75 73	254 252 245 302	356 362 358 408
III	H-150 B-130 T-160 C-120	Standard Glibenclambide 600µg/kg i.p	77 76 75 74	221 246 289 228	68 67 68 70 91 92
IV	H-140 B-150 T-130 C-140	Sample 200 mg/ kg .p.o	78 77 74 79	256 262 323 354	90 88 91 92

In conclusion, an attempt has been made to evaluate one of the medicinal plants that have attracted considerable global intrest in recent years. It is clearly found that the extract of *cocos nucifera* possess compounds with antidiabetic activity

From the study and with previous literature survey of [13],[14],[15], Obviously we conclude that the leaves of *Syzyium cumini, Aegle marmelos* and endocarpic membrane of *Cocos nucifera* is a good source which posses potent anti diabetic activity. There was a significant difference between methanol and aqueous plant extract. Consequently, the overall effects of these plant extracts cannot be fully duplicated with the isolated compounds or extracts. Because of its inherent botanical and biochemical complexity, plant extracts like bael, jambul and tender coconut had, so far be eluded as in modern science. The plant studies here can be seen as a potential source of useful drugs.

Anti diabetic activity in in vivo analysis, diabetic was induced by alloxan. It is then treated with standard drug and the plant extract. Decreased in hypoglycemic action takes place. The drugs which are given are glibenclamide and aqueous extract of endocarpic tender coconut. Moreover equal decreased in blood sugar level takes place,
Glibenclamide = 70 mg/ml;
Cocos nucifera = 88 mg/ml

The endocarps of Cocos nucifera are discarded as waste and it is considered as one of the major agro wastes of the tropical countries. Therefore our study will definitely open up a scope for future utilization of these agro wastes for

therapeutic purpose. This could be sure that the plant source were of less side effects. Soon it could reach for the treatment by the consumption of humans. Like wise [16] demonstrated the agrowaste of coconut protein has potent antidiabetic activity.

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