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EVALUATION OF POLYHERBAL ORAL FORMULATION FOR ANTIDIABETIC ACTIVITY

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ABSTRACT

The individual extracts of leaves of *Murraya koenigii* (MK), *Annona squamosa* (AS), and roots of *Plumbago zeylanica* (PZ) had shown promising results in oral glucose tolerance test (OGTT) model. In order to study the potential synergistic activity of all these herbs, the capsules of each containing 200mg extract of each of MK and AS and 40 mg of root powder of PZ were prepared and tested for antidiabetic activity in STZ induced diabetic rat model. The aqueous extracts of all the ingredients i.e. leaves of MK, AS, and root powder of PZ were prepared by hot decoction method and extracts were evaporated to dryness. The optimized dose revealed from OGTT study was selected for capsule formulation. All the ingredients of the capsules were mixed together in required proportion with suitable excipients and filled into the capsules. The capsules were studied for their physicochemical stability, content of phytochemical marker by HPTLC and their antidiabetic activity using STZ induced diabetic rat model. The OGTT study of individual extracts had revealed that at the dose of 200mg for MK and AS, lowered blood glucose by 60%. The dose of polyherbal formulation was calculated by extrapolation of animal dose to human dose. The current study showed that the polyherbal formulation was 64% as effective as Glibenclamide in STZ induced diabetic rat model. All the constituent herbs of capsule contain rutin which is use as marker compound for stability study. The capsule formulation was found to be stable over the period of six months.

Key words: *Murraya koenigii, Annona squamosa, Plumbago zeylanica*, OGTT, Antidiabetic activity.

INTRODUCTION

Diabetes mellitus is endocrine disorder causes due to loss of glucose level imbalance. The lack of insulin action and/or insulin secretion which results in impaired carbohydrate, fat and protein metabolism (Umesh *et al.,* 2004) (Sophia, 2007). The complications associated with Diabetes mellitus are increased levels of cholesterol, triglycerides, phospholipids and alterations in lipoprotein composition (WHO, 1985) (WHO, 1980). There are several plants mentioned in Indian traditional system of medicine for the treatment of diabetes mellitus (Grover *et al.,* 2002) The literature survey revealed that plants like Gudmar (*Gymnema sylvestre*), Karela (*Momordica charantia*), Guar gum (*Cyamopsis tetragonolobus*), Curry leaves (*Murraya koenigii*), *and Annona squamosa*, Chitrak *(Plumbago zeylanica)* and rajmah (*Phaseolus vulgaris*) have antidiabetic potential (Srinivasan, 2005). The present paper deals with the aim of evaluating the antidiabetic potential of extracts of *Murraya koenigii* (Arulselvan *et al.,* 2006), *Annona squamosa* (Gupta *et al.,* 2005) and *Plumbago zeylanica* (Muftah *et al.,* 2010) on blood glucose level by OGTT study and effects of polyherbal capsule formulation by studying antidiabetic activity in streptozotocin induce diabetic rat model. The stability study of formulation was also done at different time interval by HPTLC method of analysis.

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MATERIALS AND METHODS

Plant Material

The leaf drug of *Annona squamosa*, *Murraya koenigii and roots of Plumbago zeylanica* were procured from local market of Mumbai, Maharashtra. The samples were authenticated by Dr. Harshad M. Pandit, Department of Botany, Guru Nanak Khalsa College, Matunga,

Mumbai 400 019. The authentication specimen number was KAS/91a.

Chemicals

Glibenclamide tablet (5mg) was purchase from Aventis Pharma, Streptozotocin was purchase from Sisco Research Laboratories Pvt. Ltd. Mumbai, India, Glucose, Digital glucometer was purchase from Johnson and Johnson, and all other chemicals used were of analytical grade.

Preparation of Plant Extract

The leaf powder of *Murraya koenigii*, *Annona squamosa* and root powder of *Plumbago zeylanica* individually were boiled with distilled water, keeping the drug to water ratio 1:10 w/v. The extract was filtered and filtrate was evaporated to obtain dry powder. The dried extract was stored in airtight container in the refrigerator for further use.

Phytochemical screening

The aqueous extracts of *Annona squamosa*, *Murraya koenigii and Plumbago zeylanica* were subjected for preliminary phytochemical investigation (Khandelwal, 2010). The extracts were studied for phytochemical analysis given in table no.1.

OGTT study of Extracts

The oral glucose tolerance test was performed in overnight fasted normal rats (Tembhurne, 2009). Rats divided into control group, standard drug treated group and extract treated groups (n=6). The groups were administered drinking water, glibenclamide (5 mg/ kg body weight) and the crude extracts respectively. Glucose was fed after the administration of extracts. Blood was withdrawn from the retro orbital plexus, collected just prior to glucose administration (0 minute) and 30, 60, 90, 120 and 180 minute after glucose loading and the blood glucose levels were measured by glucometer. Various dose levels of the extract and fractions were titrated to finalize the therapeutic dose for further studies. The result of OGTT study was given in table no.2, 3 & 4.

Preparation of Polyherbal Formulation

The proportion and amount of each extract per unit dosage form was finalized based on their antidiabetic potential of the extracts as revealed in OGTT studies. The composition of excipents was optimized to get optimized formula mention in table no 5. The extracts and excipents were triturated together to get uniform powder mixture and was filled in the capsule shell. The capsules were standardized with respect to phytochemicals and tested for antidiabetic activity.

Evaluation of antidiabetic activity of polyherbal formulation

Animals

Adult healthy Sprague Drawly rats weighing 200-220 g obtained from Glenmark Research Centre, Mumbai were used for the study. The animals were housed in polypropylene cages under standard husbandry conditions (12 hrs light/dark cycle: 25 ± 3 °C). Rats were fed with standard rat pellet diet and water *ad libitum*. The study was conducted after the approval from the Institutional Animal Ethical Committee.

Induction of Diabetes

All the rats were given a period of acclimatization for 15 days before starting of the experiment Rats were fasted for 12-h before diabetes was induced using Streptozotocin (Adolfo *et al.*, 2000) (Choubey *et al.*, 2010). Streptozotocin (STZ) was freshly dissolved in 0.1 M citrate buffer (pH 4.5) at the dose of 60 mg/kg body weight and injected intraperitoneally within 2 minute of dissolution in a vehicle volume of 0.5 mL with 1 mL of tuberculin syringe fitted with 26 gauge needle. Serum glucose level was estimated before induction of diabetes. Diabetes was confirmed by the determination of fasting glucose concentration on the seventh and fourteenth day post administration of STZ. The rats with fasting serum glucose levels > 200 mg/dl on the 14th day post induction were considered as diabetic rats (Kesari *et al.*, 2005).

Experimental Study design

Rats were divided into the following groups (n=6), and dosed daily for 21 days, once a day.

Group I: Consisted normal rats which served as normal control and were given only distilled water.

Group II: Consisted of STZ induced diabetic rats and served as diabetic control and were given distilled water only.

Group III: Consisted of STZ induced diabetic rats and were treated orally with capsule at lower dose at 100 mg/kg body weight.

Group IV: Consisted of STZ induced diabetic rats and were treated orally with capsule at higher dose at 200 mg/kg body weight.

Group V: Consisted of STZ induced diabetic rats and were given Glibenclamide (GBC) suspended in 1% CMC at the dose of 5 mg/kg body weight.

Group VI: Consisted of STZ induced diabetic rats treated orally with 1% Carboxy methyl cellulose (CMC) and served as vehicle control.

Statistical analysis

The results were expressed as mean ± S.E.M., the significant of various treatments were calculated using

students t-test and were considered statistically significant when $p < 0.05$.

Stability Study of Formulation by HPTLC analysis

The stability study was performed by storing the capsules in the sealed amber colored glass container at 25ºC/60 RH. The batches were analyzed at 0, 3 and 6 months using HPTLC method.The capsule formulation was evaluated by HPTLC fingerprinting using CAMAG LINOMAT V applicator with 100 µl Hamilton syringe. The chromatogram was developed on silica gel F_{254} precoated HPTLC plates using Ethyl acetate: Formic acid: Glacial acetic acid: Water (10:1.1:1.1:2.6) and using UV 254 as detecting agent. The plates were scanned by using CAMAG Scanner 3 using WINCAT software. This chromatographic pattern used as standard fingerprinting data for stability studies.

Sample preparation

- 0.6 g of capsule powder was dissolved in 10 ml of Methanol.
- Sonicated the sample for 5 min and filter.
- Make up volume of filtrate to 10 ml with methanol.
- Evaporate the filtrate into evaporating dish
- Add 1ml of methanol to the extract and used for further study.

Solvent system

Ethyl acetate : Formic acid : Glacial acetic acid : Water (10:1.1:1.1:2.6) Standard used: Rutin Std.

Figure 1. Antidiabetic activity graph

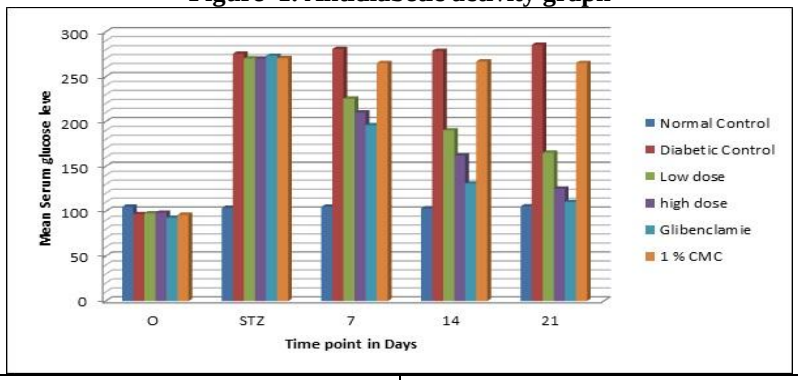
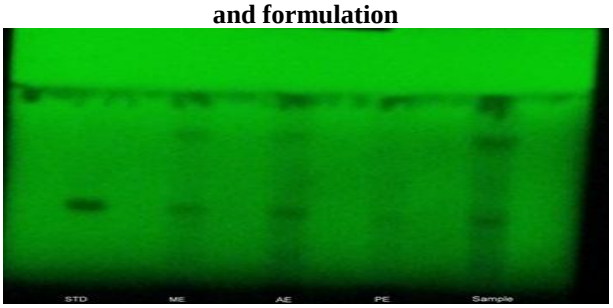


Figure 2. HPTLC plate at 254 nm showing Std, extracts and formulation



Std = Standard Rutin, ME = *Murraya* extract, AE = *Annona* extract, PE = *Plumbago* extract Sample = capsule formulation

Figure 3. 3D graph of HPTLC plate

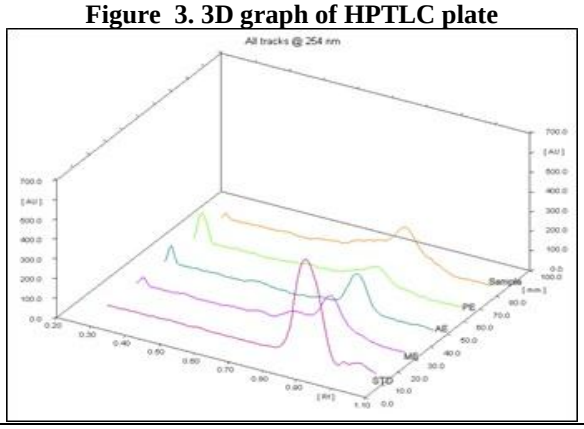


Figure 4. HPTLC plate at 254 nm showing STD and sample at 0th month

Figure 5. 3D graph of HPTLC plate

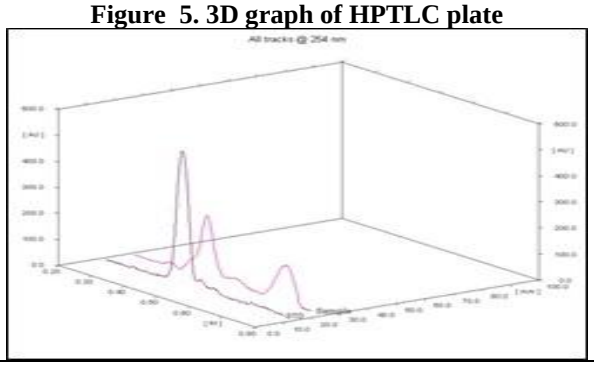


Figure 6. HPTLC plate at 254 nm showing STD and sample at 3rd month

Figure 7. 3D graph of HPTLC plate

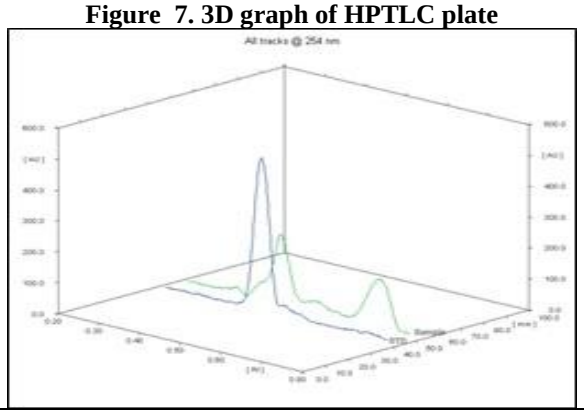


Figure 8. HPTLC plate at 254 nm showing STD and sample at 6th month

Figure 9. 3D graph of HPTLC plate

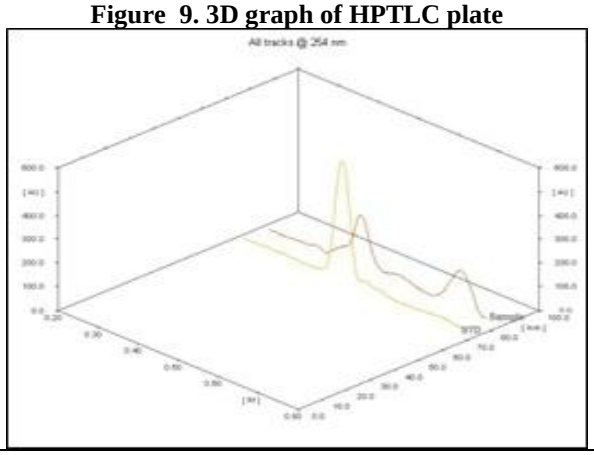


Table 1. Preliminary Phytochemical analysis of extract of leaves of *Murraya koenigii*, *Annona squamosa* and roots *Plumbago zeylanica*

Test	*Murraya koenigii* Aqueous Extracts	*Annona squamosa* Aqueous Extracts	*Plumbago zeylanica* Aqueous Extracts
Tests for Carbohydrates			
Molisch Test	+	+	+
Fehling's Test	+	+	+
Test for Alkaloids			
Dragendorff's Test	+	+	-
Tests for Cardiac glycosides		-	-
Legal's Test	-	-	-
Keller-Killiani Test	-		
Tests for Anthroquinone glycosides		-	
Borntrager's Test	-	-	-
Modified Borntrager's Test	-		-
Test for Saponin glycosides		-	
Foam Test	-		+
Test for Flavonoids		+	
Lead acetate Test	-		+
Tests for Phenolics		+	+
5 % FeCl3 solution	+	+	+
Bromine Water Dilute $KMnO_4$ solution	+ +	+	+

+ Present, - Absent,

Table 2. OGTT study result of Aqueous extract of *Murraya koenigii*

Time (minute)	Mean % Increase in Serum Glucose Level ± SEM				
	Control Group	Glibenclamide Treated Group	Aqueous Extract Treated Groups of *Murraya koenigii*		
			100mg/kg b.w.	250mg/kg b.w.	500mg/kg b.w.
0	-	-	-	-	-
30	58.96 ± 2.14	1.46 ± 0.10	35.81 ± 1.57	48.17 ± 3.53	61.68 ± 3.66
60	100.40 ± 3.16	2.06 ± 0.14	52.64 ± 1.32	63.39 ± 3.23	74.65 ± 4.18
90	78.61 ± 2.45	2.21 ± 0.17	41.13 ± 2.08	43.50 ± 3.69	56.23 ± 4.47
120	68.55 ± 3.65	2.29 ± 0.19	36.36 ± 2.36	32.59 ± 2.56	42.30 ± 3.27
180	34.93 ± 4.12	3.67 ± 0.20	18.32 ± 1.49	24.94 ± 2.48	24.11 ± 1.78
P value with control	-	0.0118	0.0125	0.0237	0.0484

Table 3. OGTT study result of aqueous extract of *Annona squamosa*

Time (minute)	Mean % Increase in Serum Glucose Level ± SEM				
	Control Group	Glibenclamide Treated Group	Aqueous Extract Treated Groups of *Annona squamosa*		
			100mg/kg b.w.	200mg/kg b.w.	400mg/kg b.w.
0	-	-	-	-	-
30	58.96 ± 2.14	1.46 ± 0.10	63.71 ± 2.87	76.53 ± 1.34	88.30 ± 1.26
60	100.40 ± 3.16	2.06 ± 0.14	64.52 ± 1.32	68.50 ± 1.54	83.30 ± 1.54
90	78.61 ± 2.45	2.21 ± 0.17	62.47 ± 1.93	68.27 ± 2.23	65.92 ± 2.34
120	68.55 ± 3.65	2.29 ± 0.19	55.17 ± 2.24	60.87 ± 1,07	52.67 ± 2.89
180	34.93 ± 4.12	3.67 ± 0.20	10.35 ± 2.29	12.04 ± 2.35	5.30 ± 1.89
P value with control	-	0.0118	0.0237	0.0484	0.0125

Table 4. OGTT study result of Aqueous extract of *Plumbago zeylanica*

Time (minute)	Mean % Increase in Serum Glucose Level ± SEM			
	Control Group	Glibenclamide Treated Group	Aqueous Extract Treated Groups of *Plumbago zeylanica*	
			50mg/kg b.w.	100mg/kg b.w.
0	-	-	-	-
30	58.96 ± 2.14	1.46 ± 0.10	57.23 ± 2.12	54.01 ± 2.32
60	100.40 ± 3.16	2.06 ± 0.14	50.90 ± 1.62	40.15 ± 3.14
90	78.61 ± 2.45	2.21 ± 0.17	34.41 ± 2.32	24.74 ± 2.71
120	68.55 ± 3.65	2.29 ± 0.19	27.22 ± 1.86	8.60 ± 2.38
180	34.93 ± 4.12	3.67 ± 0.20	18.52 ± 2.12	2.97 ± 2.13
P value with control	-	0.0118	0.0234	0.0154

Table 5. Optimized formula for Capsule formulation

Ingredient	For 1 capsule (Wt in mg)
Murraya aq. Ext	200
Annona aq. Ext	200
Plumbago zeylanica root powder	40
Lactose	170
Total wt	610
Silicon dioxide was added 1% of total weight	

Table 6. Details of the Antidiabetic activity graph (Time point in day Vs mean serum glucose level)

Sr. No.	Normal Control	Diabetic Control	Low dose (100 mg/kg body wt)	High dose (200 mg/kg body wt)	Glibenclamide	1 % CMC
O	105.09	96.85	97.6	98.41	92.72	95.99
STZ	103.7	275.53	270.28	270.12	273.25	270.49
7	104.93	280.88	225.58	210.03	195.76	265.23
14	103.2	278.65	190.15	162.29	130.83	267.03
21	105.49	285.36	165.34	125.15	110.35	265.2

RESULTS AND DISCUSSION

The yield of extract was found to be 20±2%w/w, 19.4±2%w/w and 23.1±2%w/w of *Annona squamosa, Murraya koenigii and Plumbago zeylanica* respectively. The preliminary phytochemical analysis of aqueous extracts of *Annona squamosa, Murraya koenigii and Plumbago zeylanica* shows presence of Carbohydrate, phenolic, flavanoids, alkaloids, carbazole alkaloids, protein, resins, coumarine, Beta-sitosterol and naphthoquinone derivatives etc., as shown in table no.1.

The preliminary OGTT study results shows a percentage increase in serum glucose level in control group was about 100 % at 60 minute after glucose loading. The aqueous extract of *Murraya koenigii*, *Annona squamosa* at dose of 200 mg each are efficient in reducing the blood glucose level by about 64%. Accordingly extrapolation of human dose it comes to 1.5 gm of divided dose. *Plumbago zeylanica* extract also has shown promising antidiabetic potential at very low dose ranges. So we decided to incorporate root powder of *Plumbago zeylanica* in formulation. Hence, the capsules were prepared with each containing 200 mg of aqueous extract of *Murraya koenigii* and aqueous extract of *Annona squamosa* of each and 40 mg of root powder of *Plumbago zeylanica*. The optimized formula was shown in table no 5.

The results of antidiabetic study reveal that polyherbal formulation shows dose dependent protection against increase in glucose level when compared with that of control group. Low dose of extract, high dose of extract and standard treatment showed 69, 27 & 17% increase in glucose levels respectively as compared to control group which shows194% increase in glucose level. Hence, the formulation was 64% as effective as Glibenclamide in STZ induced diabetic rat model. The study has thus resulted in preparation of stable oral herbal dosage form with proven efficacy for management of diabetes.

The HPTLC analysis of capsule formulation was done with respect to Rutin as standard compound because rutin was found to be main marker compound present in all extracts as shown in fig no. 2 and 3 Hence, based on this finding the stability study of capsule formulation was carried out by HPTLC analysis at three different time interval of 0^{th} month, 3^{rd} month and 6^{th} month as shown in fig No. 4 to 9. The HPTLC chromatogram was reveals that the capsules are stable when tested up to six months.

CONCLUSION

The current paper helps us to explore the therapeutic potential of some of the indigenous herbs namely *Murraya koenigii, Annona squamosa* and *Plumbago zeylanica* for management and control of diabetes. These herbs have shown very good antidiabetic potential. The prepared oral dosage form containing all the three herbs to take advantage of their synergistic effect. This indicates that the present formulation has excellent protective effect against increase in glucose level in Streptozotocin induced diabetes model.

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