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"STUDY OF HYPOGLYSEMIC EFFECT OF SOME FLOWER'S PETALS EXTRACT & THEIR FORMULATIONS"

H. B. Waghire **M.A.KARE,S.A.PATIL AND SHETE H.B,**
*MJS MAHAVIDYALAYA SHRIGONDA 413701
**PRATISTHAN MAAHAVIDYALAYA PAITHAN
***S.S.G.M COLLEGE KOPERGAON DIST. AHMEDNAGAR (M.S.) 423601

ABSTRACT

Present paper has studied hypoglycemic effect of different flowers.These study demonstrated that several flowers were able to influenced α-amylase activity. Out of these, *Withania coagulans* was proved significant for inhibiting α-amylase activity. In present investigation, fourteen flowers were tested, out of these one can have marked hypoglycemic effect by inhibiting the α-amylase.

Key world: *Amylase,Withania Catharanthus rosus.*

INTRODUCTION

Diabetes mellitus is a complex disease characterized by defects in carbohydrates, fats and protein metabolism [1]. It results from the defects in insulin secretion, action or in both [2]. D.M. is a serious health problem being the third greatest cause of death all over the world, and if not treated, it is responsible for many complications affecting the various organs in the body [3]. The rate of diabetes is increasing. Worldwide, it affects 230 million people of which 30 millions are from India [2]. Diabetes mellitus is classified into different categories, based on the etiology of disease, but two main types are widely accepted. viz D.M. Type -1,occurs in patients with little or no insulin secretary capacity and D.M.Type -2,is most known form, characterized by abnormality in insulin secretion and its resistance[4].Mammalian α-Amylase is a prominent enzyme in pancreatic juice which breaks down large and insoluble starch molecules in to absorbable molecules[1]. The most important digestive enzyme is pancreatic α-Amylase catalyzes the hydrolysis of α-1, 4 glycosidic linkages of starch, amylose, amylopectin, and glycogen and is responsible of most of starch digestion in humans [2]. The common strategy is regulating and decreasing the blood sugar level to fall within the normal level [4]. One effective way of tackling the problem is to inhibit the activity of digestive enzymes like α-Amylase and others that are involved in the hydrolysis of starch hence reducing the concentration of sugar after meals in the diabetic patients [8].The management of diabetes can be achieved by reducing post-prandial hyperglycemia by delaying the activities of the enzyme α- Amylase and α- glycosidase which are responsible for the digestion of carbohydrates and absorption of glucose in the digestive tract, respectively [5].

Medicinal plant as a source of remedies, are widely used as alternative therapeutic tools for the prevention or treatment of many disease. Recently great attention has been devoted to the use of natural compound, due to their nutritional and pharmacological characteristics[6].Different plants have been reported to show α-Amylase inhibitory activity Chemical inhibitor of α-Amylase and other

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carbohydrate digestive enzymes are known to produce serious side effects that limit their use as a therapeutic drug[2].Existing hypo glycemic agents such as metformin,voglibose,acarbose and miglitol effectively control glycemic level but carry prominentgastrointestinal effects. The search for inhibitors devoid of side effects has been geared towards natural resources, namely, medicinal plants [10]. Herbal medicines have ever been used and claimed as antidiabetic agents but very less are available on commercially formulated forms [7]. Plants are well known in traditional medicine for their hyperglycemic activities. There has been increasing demand for the use of plant products with antidiabetic activity due to low cost, easy availability and lesser side effects. Therefore, plants are being continuously explored for their possible effect as hypoglycemic agent [9].

REVIEW OF LITERATURE

Mishra and Vijayakumar (2014) evaluated the antihyperglycemic and antioxidant effect of 50% ethanolic extract (200 mg/kg body weight) of *Saraca asoca* flowers in streptozotocin-nicotinamide induced diabetic rats. The extract was found to reduce blood glucose levels as well as normalized the decreased activities of key antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione in diabetic rats.Prathapan et al., (2012) investigated the inhibitory potential of flavonoid fraction of Saraca asoca flowers (SAF) against α-glucosidase and α-amylase and LDL oxidation and found significant inhibitory potential of SAF against these enzymes there by proving its antihyperglycemic activity. They also found that pre-treatment of C2C12cells with SAF prevented the increased formation of MDA and depletion of GSH induced by H2O2. SAF also demonstrated potent antiglycation property and inhibited LDL oxidation under in vitro conditions.Pal et al., (2012) evaluated the free radical scavenging activity were measured in the ethanolic and water extract of fresh and dried flowers of Saraca indica. Free Radical scavenging activities of the extracts were evaluated using DPPH assay method and were found to be higher in fresh flower extract (both ethanol and water) in comparison to dried flower. Somani et al., (2015) investigated SI flowers and its fractions as a potential Aldose Reductase (AR) enzyme inhibitor and preventing cataractogenesis in goat lens model (in vitro), followed by in vivo evaluation of streptozotocin (STZ)-induced cataract formation in diabetic rats. Ethyl acetate fraction showed highest percentage of inhibition (IC50=4.69 µg/ml) followed by methanol extract 13.54 µg/ml. The ethyl acetate fraction was subjected to various analytical techniques such as HPTLC, HPLC and LC-MS which revealed the presence of gallic acid, anthocyanidin, pelargonidin-3, 5-diglucoside, cyanidin-3, 5-diglucoside and β-sitosterol.Pradhan et al., (2010) studied the pharmacognostical feature, including macroscopy, microscopy and analytical profile and also investigated the phytochemical and quantitative determination of phytoconstituents namely tannins, flavonoids and saponins from the leaves of *Saraca asoca*.

Saha *et al*., (2012) developed a reliable and sensitive HPTLC method for qualitative determination of gallic acid in the dried flowers and leaves of *Saraca asoca* (Roxb.)Wild. The separation was achieved on precoated silica gel 60 F254 HPTLC plates as stationary phase and toluene: ethyl acetate: formic acid: methanol (6:6:1.6:0.4 v/v/v/v) as mobile phase at λ 280 nm for gallic acid. The presence of gallic acid is reported for the first time in dried flowers and leaves by the authors.

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Rout *et al*., (2010) performed comparative analysis of the fragrance obtained from Mimusops elengi flowers by different methods viz. water soluble volatiles, hexane extract and liquid CO2 extract. The extracts were analyzed by GC and GC/MS. The chemical compositions of the extracts obtained were rich in bezenoids (61.7%), being phenyl ethyl alcohol (23.6 32.5%) the major compound. Nagakannan et al., (2012) carried out simultaneous quantification of five polyphenolic biomarkers from ethanol extract of dried flowers of the plant using HPLC analysis. Polyphenols present in the ethanol extract were, protocatechuic acid (0.47±0.02 mg/g), chlorogenic acid (0.36±0.01 mg/g), caffeic acid (0.40±0.03 mg/g), rutin (1.12±0.03 mg/g) and luteolin-7-O-glucoside (0.19±0.02 mg/g).

Materials & Methods :-

Flowers Used -

Scientific Name	Common Name	Family
1. *Tabernaemontana divaricata*	Crape jasmine	Apocynaceae
2. *Gaillardia pulchella*	Galanda	Asteraceae
3. *Nymphaea pubescens*	Water lily	Nymphaeaceae
4. *Caesalpinia pulcherrima*	Peacock flower	Fabaceae
5. *Ixora coccinea*	Ixora	Rubiaceae
6. *Tagetes erecta*	Marigold	Asteraceae
7. *Rosa indica*	Rose	Rosaceae
8. *Pterospermum acerifolium*	Kanak champa	Sterculiaceae
9. *Withania coagulans*	Paneer phool	Solanaceae
10. *Hibiscus rosa-sinensis*	Hibiscus	Malvaceae
11. *Chrysanthemum morifolium*	Crysanthemum	Asteraceae
12. *Symphyotrichum lanceolatum*	Aster	Asteraceae
13. *Catharanthus rosus*	Periwinkle	Apocynaceae
14. *Nycanthus arbor-tritis*	Parijat	Oleaceae

Formulations of Flowers :-

Common Name	Quantity (ml)
1. Paneer phool + Hibiscus	1 : 1
2. Paneer phool + Hibiscus + Kanak champa	1 : 1 : 1
3. Paneer phool + Periwinkle + parijat	1 : 1 : 1
4. Paneer phool + Rose	1 : 1

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5. Paneer phool + Crysanthemum + Kanak champa	1 : 1 : 1
6. Paneer phool + Parijat + Crape jasmine	1 : 1 : 1
7. Paneer phool + Crape jasmine	1 : 1
8. Paneer phool + Periwinkle + Hibiscus + Parijat	1 : 1 : 1 : 1
9. Paneer phool + Hibiscus + Rose	1 : 1 : 1
10. Paneer phool + Crape jasmine + Hibiscus + Periwinkle + Parijat	1 : 1 : 1 : 1 : 1
11. Paneer phool + Water lily + Peacock flower + Galanda	1 : 1 : 1 : 1
12. Paneer phool + Crape jasmine + Hibiscus + Rose	1 : 1 : 1 : 1

Preparation of weed extract-

- Fresh, healthy and disease free plant material was collected from field, brought to laboratory, and washed thoroughly.5 gm of flower were taken. Then grinded in mortar with the help of pestle using distilled water so as to obtain aqueous extract. This aqueous extract then used for further assay.

Preparation of salivary amylase-

- 10 ml of saliva was diluted twice by adding phosphate buffer of pH-7 .
- The diluted saliva was used as source of α-amylase.

Preparation of starch-agar gel-

- 600 ml water was boiled.
- 6 gm agar powder and 1000 mg starch powder was added to the boiling water.
- The solution was stirred continuously till it become colorless.

Preparation of reaction mixture-

- 2.5 ml of plant extract was mixed with 50 ml of starch-agar gel along with 2 ml buffer solution.
- This reaction mixture was poured in Petri plates with respective plant extract used.
- The reaction mixture for control was prepared using starch-agar gel with buffer without any plant extract.
- This mixture was allowed to solidify.
- Wells are bored with the help of borer.
- Bottom of each well was sealed using starch agar gel.
- Each petriplate was labeled with name of respective reaction mixtures.
- Then 2.5 ml of diluted saliva was loaded in the wells.
- Sets were allowed to incubate overnight.

Treatment of KI-

- After incubation, plates were flooded with aq. solution of KI.
- After 5 minutes, solution was removed.
- Then zone of digestion was observed and measured

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OBSERVATION TABLE :-

Petri plate	Zone Of Digestion (cm)
1	6.2
2	6.4
3	6.3
4	6.6
5	6.5
6	6.4
7	6.8
8	6.2
9	5.6
10	5.8
11	6.4
12	6.4
13	5.9
14	6.0
Control	7.0

Calculation by inhibition test :

%amylase activity = $\frac{\text{Diameter of test circle}}{\text{Diameter of control circle}} \times 100$

% Inhibition = 100 - amylase activity.

RESULT & DISCUSSION :

STARCH AGAR DIFFUSION ASSAY :

NAME OF FLOWER PLANT	ZONE OF DIGESTION(cm)	% DIGESTION	% INHIBITION
1) *Tabernaemontana divaricata*	6.2	88.57	11.43
2) *Gaillardia pulchella*	6.4	91.42	08.58
3) *Nymphea pubescens*	6.3	90.00	10.00
4) *Caesalpinia pulcherrima*	6.6	94.28	05.72
5) *Ixora coccinea*	6.5	92.85	07.15
6) *Tagetes erecta*	6.4	91.42	08.58
7) *Rosa indica*	6.8	97.14	02.86
8) *Pterospermum acerifolium*	6.2	88.57	11.43
9) *Withania coagulans*	5.6	80.00	20.00
10) *Hibiscus rosa-sinensis*	5.8	82.85	17.15
11) *Crysanthemum morifolium*	6.4	91.42	08.58
12) *Symphotrichum lanceolatum*	6.4	91.42	08.58

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13) *Catharanthus rosus*	5.9	84.28	15.72
14) *Nycanthus arbor-tritis*	6.0	85.71	14.29
15) Control	7.0	-	-

OBSERVATION TABLE :-

Petri plate	Zone Of Digestion (cm)
1	**5.5**
2	**5.5**
3	**5.4**
4	**6.0**
5	**5.7**
6	**5.5**
7	**5.7**
8	**5.4**
9	**5.8**
10	**5.4**
11	**5.7**
12	**5.6**
Control	**7.0**

Calculation by inhibition test :

- %amylase activity = $\frac{\text{Diameter of test circle}}{\text{Diameter of control circle}} \times 100$
- % Inhibition = 100 - amylase activity.

RESULT & DISCUSSION :-
STARCH AGAR GEL DIFFUSION ASSAY -

Common Name	Zone of Digestion(cm)	% Digestion	% Inhibition
1) Paneer phool + Hibiscus	5.5	78.57	21.43
2) Paneer phool + Hibiscus + Kanak champa	5.5	78.57	21.43
3) Paneer phool + Periwinkle + parijat	5.4	77.14	22.86
4)Paneer phool + Rose	6.0	85.71	14.29
5) Paneer phool + Crysanthemum + Kanak champa	5.7	81.42	18.58

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6)Paneer phool + Parijat + Crape jasmine	5.5	78.57	21.43
7)Paneer phool + Crape jasmine	5.7	81.42	18.58
8)Paneer phool + Periwinkle + Hibiscus + Parijat	5.4	77.14	22.86
9)Paneer phool + Hibiscus + Rose	5.8	82.85	17.15
10)Paneer phool + Crape jasmine + Hibiscus + periwinkle + Parijat	5.4	77.14	22.86
11)Paneer phool + Water lily + Peacock flower + Galanda	5.7	81.42	18.58
12)Paneer phool + Crape jasmine + Hibiscus + Rose	5.6	80.00	20.00
13)Control	7.0	-	-

DISCUSSION :-

Our study demonstrated that several flowers were able to influenced α-amylase activity. Out of these, *Withania coagulans* was proved significant for inhibiting α-amylase activity. In present investigation, fourteen flowers were tested, out of these one can have marked hypoglycemic effect by inhibiting the α-amylase. Therefore these flower extract could offer a good potential to treat diabetes.

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