



ISSN NO. 2320-5407

Journal homepage: <http://www.journalijar.com>  
Journal DOI: [10.21474/IJAR01](https://doi.org/10.21474/IJAR01)

INTERNATIONAL JOURNAL  
OF ADVANCED RESEARCH

## RESEARCH ARTICLE

**Phytochemical composition and *in-vitro* Anti-diabetic potential of *Swietenia mahagoni* seed pod.**

**Naveen Y P<sup>1</sup>, kalpashree K M<sup>1</sup>, Banushree B<sup>1</sup> and Jyothi bala Chauhan<sup>1</sup>.**

Department of Biochemistry, Pooja Bhagavath mahajana PG center, Mysore-16.

**Manuscript Info**

**Manuscript History:**

Received: 15 May 2016  
Final Accepted: 19 June 2016  
Published Online: July 2016

**Key words:**

*Swietenia mahagoni*, anti-diabetic, seed pod, extract.

**\*Corresponding Author**

**Naveen Y P.**

**Abstract**

*Swietenia mahagoni* seed pod was screened for its anti-hyperglycemic potential by using *In-vitro* assays. Dried powder of seed pod was used in glucose adsorption and glucose diffusion retardation index assay, where as the aqueous and methanol extract of the seed were used to study the inhibition of enteric enzymes ( $\alpha$ -amylase and sucrase). The bark powder showed dose dependent glucose adsorption and glucose diffusion retardation index. Aqueous extract was more potent in inhibiting the activity of  $\alpha$ -amylase than methanol extract. There was no notable activity observed in case of sucrase assay. Overall, assays partly indicate the anti-hyperglycemic potency of the seed pod which may be mediated through the inhibition of enteric enzymes. Further investigations are needed to validate this observation

Copy Right, IJAR, 2016.. All rights reserved.

**Introduction:-**

Medicinal plants have shown to ameliorate diabetic conditions by acting as enteric carbohydrate hydrolyzing enzyme inhibitors, glucose uptake promoters, insulin sensitizers, insulin potentiating agents, anti-oxidants, gluconeogenesis inhibitors, anti-hypercholesterolaemic agents etc (Grabley and Thiericke, 1999). Many medicinal plants with the promising potential are being subjected to clinical studies for their complete validation as anti-diabetic agents or adjuvants. Among the thousands of explored medicinal plants only a few are clinically validated in human subjects. Medicinal plants such as *Gymnema sylvestre*, *Cinnamon cuminum*, *Allium sativum*, *Aloe vera*, *Artocarpus heterophyllus*, *Morus Indica*, *Asteracanthus longifolia*, *Bauhinia forficata*, *Coccinia indica*, *Ficus carica*, *Ficus raecemosa*, *Panax quinquefolius*, *Myrcia uniflora*, *Ocimum sanctum*, *Opuntia streptacantha*, *Trigonella foenum*, *Asteracantha longifolia* etc., have been tested for their anti-diabetic potency in human subjects (Shane-McWhorter, 2001; Lucy *et al.*, 2002; Sheela and Augusti, 1992).

Medicinal plants and formulations comprising many medicinal plants are being promoted as anti-diabetic drugs in the drug market. Although there is an intense research on the development of herbal drugs, only a small fraction of medicinal plants have been explored and validated. In this context, there is scope for screening and development of unexplored medicinal plants as antidiabetic agents or as adjuvant. *In-vitro* and *ex-vivo* assays form a very crucial role in development of an anti-diabetic drug. Enteric enzyme inhibition assays such as  $\alpha$ -amylase inhibition, sucrase inhibition and  $\alpha$ -glucosidase inhibition are widely used preliminary assays to screen the medicinal plants for anti-diabetic potential (Krutika Thorat *et al.*, 2012).

*Swietenia mahagoni* Jacq. is a small leafy, medium sized tree native to west indies. The parts of the plant have been used to treat many human ailments such as malaria, diabetes, diarrhea, astringent, hypertension etc. locally. The fruit of the plant is used as powerful anti-hyperglycemic drug. The seed oil is being used as an alternative body ointment therapy for a range of skin cuts, itches and wounds to ameliorate the healing process in African countries. Decoction of bark is used to increase appetite, as an energizer in case of tuberculosis, to treat anemia, diarrhea, dysentery, fever and toothache. Thus, the present study was planned to evaluate the potential of *Swietenia mahagoni* seed pod using various *in-vitro* assays.

## Methods:-

### Preparation and extraction of sample:-

#### Collection and preparation of samples:-

The seed pod of the sweitenia mahagoni fruits were collected from University of Mysore campus in the month of Feb 2016.

### Sample preparation:-

The collected seed pod was thoroughly washed under running water to remove adhering dirt and other foreign particles, dried for a day at 50°C. The dried sample was powdered and passed through 60 mesh sieve. Thus obtained powder was stored in air tight container at 4°C till further use.

### Preparation of aqueous extract of *S.mahagoni* seed pod :-

The aqueous extract of the sample was prepared by extracting powder material with distilled water in a mechanical shaker for 24 h at ambient temperature. The solution obtained was filtered through Whatman filter paper (No.4) to separate insoluble component and thus obtained filtrate was freeze dried. A extract with brown colored and fine powdered texture was obtained after freeze drying.

### Preparation of methanol extract of *S.mahagoni*:-

The methanol extract of the sample was prepared by extracting powder material with methanol in a mechanical shaker for 24 h. Filtered to separate insoluble component and the filtrate was flash evaporated. The brown colored methanol extract obtained after flash evaporation was used in all *in-vitro* assays.

## Phytochemical composition:-

### Estimation of total phenolics:-

Total phenolics: Total phenolic content of the leaf was assayed by the method of Folin Ciocalteu (Slinkard and Singleton., 1977). In brief, various aliquots of aqueous methanol extract of the seed pod (10 mg/mL) were mixed with 5 mL Folin-Ciocalteu reagent and 4 mL of sodium carbonate (75 g/L). The resulting solution was vortexed and incubated at 40°C for 30 min. The absorption was read at 765 nm. A calibration curve was prepared by using gallic acid as standard. All determinations were performed in triplicate. Total content of phenolic compounds in seed pod methanol extracts in gallic acid equivalents (GAE) was calculated by the following formula:

$$A = 0.980C + 9.925 \times 10.3$$

Where,

A is the absorbance of the sample and C is the Concentration as gallic acid equivalents ( $\mu\text{g/ml}$ )

### Saponins:-

The Saponin content was analyzed according to a gravimetric method previously described (Miliauskas *et.al.*, 2004). In brief, 250 mL of 20% ethanol was added to 10 g of the pulverized bark powder and stirred at 55°C using a magnetic stirrer for 12 h. The resulting solution was filtered using filter paper (Whatman No. 1) and the filtrate volume was reduced to 40 mL under vacuum, to this 20 mL diethyl ether was added in a separating funnel and shaken vigorously. The pH of the aqueous layer was adjusted to 4.5 by adding NaOH, whereas the ether layer was discarded. The pH adjusted aqueous part was extracted with 60 mL of n-butanol. The butanol extract was washed twice with 10 mL of 5% NaCl and evaporated to dryness to give a crude saponin extract, which was weighed and expressed in g/g of the seed pod powder.

### Tannins:-

Tannins were estimated according to spectrophotometric method described by Trease & Evans (Hudson and El-Difrawi, 1979). 5 g of the seed pod powder was extracted with 50 mL of boiling water and filtered. 0.5 mL of the filtrate was added to 0.5 mL of ferric solution (0.5M) in an alkaline medium and allowed to stand for 30 min for color development. The absorbance was read at 760 nm and the amount of tannin was extrapolated from a standard calibration curve for tannic acid.

### Estimation of total alkaloids:-

Alkaloids were estimated by the gravimetric method (Naveen Y P and Asna Urooj, 2015). The sample (0.5 gm) was taken in 250 ml beaker and 200 ml of acetic acid (10%) in ethanol was added and incubated at room temperature for

10 h. The resulting solution was filtered and the extract was concentrated on a water bath to one quarter of the original volume. To the Concentrate, concentrated ammonium hydroxide was added drop wise until the precipitation was complete. The above solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and filtered. Thus resulting alkaloid precipitate was dried, weighed and expressed in alkaloids per gm of sample taken.

### Estimation of Flavonoids:-

The content of flavonoids was determined by a pharmacopeia method (Bickel H and Schultz G, 1976) using rutin as a reference compound. For brief, One ml of aqueous extract in methanol (10 mg/ml) was mixed with 1 ml aluminiumtrichloride in ethanol (20 g/l) and diluted with ethanol to 25 ml. The absorption at 415 nm was read after 40 min at 20 C. Blank samples were prepared from 1 ml plant extract and 1 drop acetic acid, and diluted to 25 ml. A standard graph was constructed using rutin as the reference standard using the above method. All determinations were carried out in triplicate. The amount of flavonoids in plant extracts in rutin equivalents (RE) was calculated by the following formula.

where, X- Flavanoids content (mg/g) plant extract in rutin equivalents, A-Absorbance of the Sample, A<sub>o</sub>-Absorbance of the standard, m- Weight of the sample in mg, m<sub>o</sub>- Weight of rutin in the solution.

### Anti-diabetic assays:-

#### $\alpha$ -amylase Inhibition Assay:-

The  $\alpha$ - amylase enzyme assay was carried out according to the procedure defined by Ou et al . Effect of seed pod aqueous and methanol extract on activity of enzymes was assayed using enzymes starch system. Various concentrations of aqueous and methanol extract and 100 mg of  $\alpha$  amylase enzyme powder added to 25ml of potato starch solution in 50 ml plastic tubes and mixed well using vortex. The tubes were incubated at 37°C for 60 min in a shaking water bath. After incubation 2ml of 0.1 M NaOH was added and heated on a boiling water bath for 10min to stop the enzymatic reaction. The tubes were cooled and liberated glucose was measured using GOD-POD method .A control was also run without adding any test sample. The amylase inhibitory activity in percentage was calculated using the below formula.

$$\% \text{ Inhibition} = \frac{\text{Abs of Control} - \text{Abs of Sample}}{\text{Abs of Control}} \times 100$$

#### Glucose adsorption assay:-

Glucose adsorption assay was carried out according to method of a previous study (Ou et.al., 2001). Seed pod powder at various concentrations (1, 2, 3 and 4 %) were added to 25 mL of glucose solution at various concentrations (5, 10, 15, 20 and 25 m mol) in 50 mL volume centrifuge tubes. The tubes were incubated on a shaking water bath for 6 h at 37°C. After incubation, tubes were centrifuged at 2500  $\times$ g for 5 min. The remaining glucose in the supernatant was measured using GODPOD method. A control was also run without adding any test samples. Glucose adsorbed by the sample was calculated by the following relation mentioned below.

$$\text{Glucose adsorbed [mmol]} = \frac{G1 - G2}{\text{Weight of the sample}} \times \text{Volume of the sample (ml)}$$

Where, G1 glucose concentration original solution and G2 is glucose concentration of the same solution after incubation for 6h.

#### Glucose uptake in the yeast cells:-

Yeast cells were prepared according to the method ou et al. Briefly, commercial baker's yeast was washed by repeating centrifugation (3,000 RPM for 5min) in distilled water until the supernatant fluids were clear and a 10%(v/v) suspension was prepared in distilled water . various concentration of aqueous and methanol extracts (1-5mg) were added to 1ml of glucose solution (5.10 and 25Mm) and incubated for 10min at 37°C reaction was started by adding 100 $\mu$ l of yeast suspension, vortex and further incubated at 37°C for 60min. After 60min the tubes were centrifuged (2,500 $\times$ g for 5min) and glucose was estimated in the supernatant.

#### Glucose diffusion retardation Index:-

Potato starch solution (1%) was prepared in phosphate buffer (0.05 M, pH 6.5). 25mL of starch solution was added with  $\alpha$ -amylase (100 mg) and seed pod powder at various concentrations in dialysis bags of 12 KD cutoff, and dialyzed against 200mL of deionized water at 37°C in a shaker water bath. The glucose diffused into the dialysate

was determined at 20,40,60 and 120 min using GOD /POD diagnostic kit. The values are indices of glucose diffusion retardation index (GDRI). A control, without samples, was also run in the same conditions.

## Results:-

### Phytochemical composition:-

The phytochemical composition of the *Swietenia mahagoni* seed pod extract is presented in the Table. From the table it can be observed the extract has a significant amount of total polyphenols, tannins and saponins.

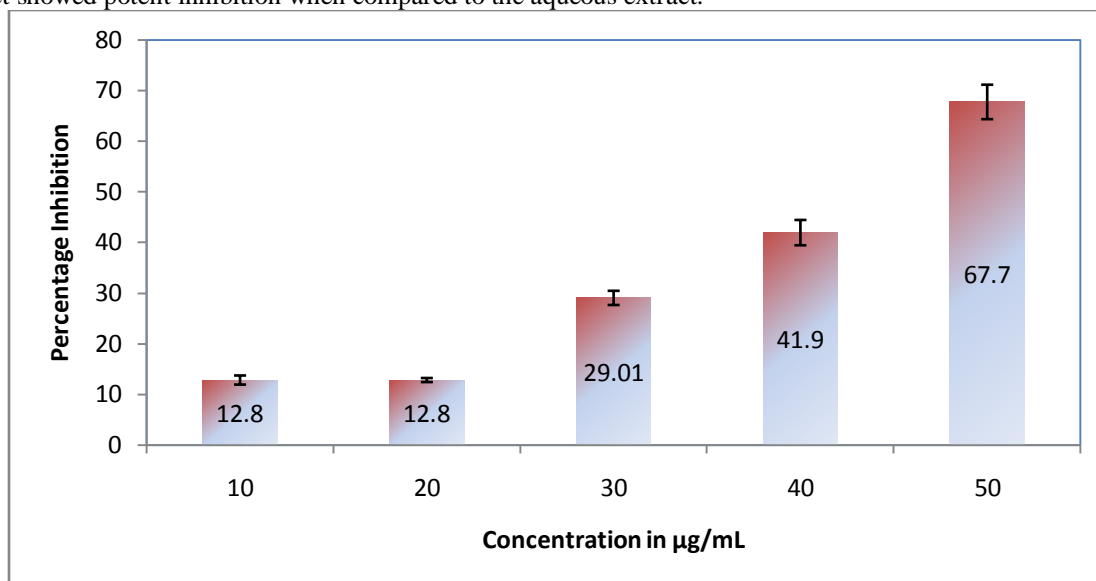
**Table 1:-** Phytochemical composition of *S. mahagoni* seed pod

Class of phytochemicals	Quantity present
Total polyphenol (mg/g)	62±0.8
Saponins (mg/g)	45±0.9
Tannins (µg/mg)	48.4±1.1
Flavonoids (µg/mg)	900±36
Alkaloids (µg/mg)	100±2.4

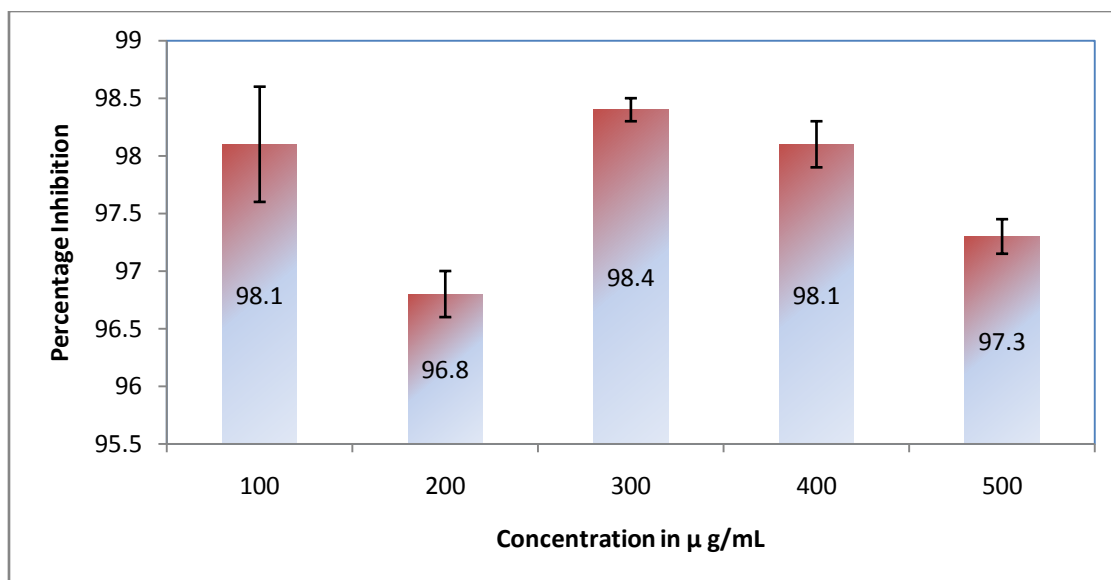
### Antidiabetic potential:-

#### Alpha amylase inhibition:-

Effect of aqueous and methanol extract on the activity of alpha amylase is given in the figure 3 and 4. Methanol extract showed potent inhibition when compared to the aqueous extract.



**Figure 1:-** Alpha amylase Inhibition by methanol extract



**Figure 2:-** Alpha amylase assay in aqueous extract

### Glucose adsorption:-

Glucose adsorbing ability of the seed pod powder is given in the table 2. The glucose adsorbing ability increases with the glucose concentration and also with increase in the sample concentration.

**Table 2:-** Glucose adsorption by the sample

Glucose in mmol	Glucose adsorbed by the sample		
	1%	2%	3%
15	0.09615	4.8398	6.7986
30	2.4319	6.5756	11.775
45	0.8595	16.6059	37.9846
60	0	5.96	10.2

### Glucose uptake in yeast cells:-

Both the extracts showed moderate inhibitory activity on the glucose uptake by the yeast cells.

### Glucose diffusion retardation index:-

Effect of the seed pod powder on glucose diffusion retardation index is given the Table 3. The glucose retardation potential increased with the increase in sample concentration.

**Table 3.** Glucose diffusion retardation index

	0 mins	20 mins	40 mins	60 mins	120 mins
Control	-0.019	0.021	0.033	0.034	0.041
1 %	-0.021	0.022	0.028	0.030	0.036
2%	-0.019	0.015	0.021	0.026	0.029
3%	0.022	0.018	0.019	0.020	0.025
4 %	0.010	0.018	0.019	0.021	0.022

### Discussion:-

Swietenia mahagoni is one of the traditional medicines used by folklore to treat various human ailments. Studies have shown that various parts of the plant are blessed with phytochemical groups having potential pharmacological value. The current study quantitates the constituent phytochemical groups in the seed pod of the plant. Polyphenols are a class of ubiquitous bio-chemicals found in most of the medicinal plants at varieng concentration. Studies have shown that the consumption of these in the form of extract reduces the risk of a number of chronic diseases such as cancer, cardiovascular disease (CVD) and neurodegenerative disorders (Gross *et.al.*, 2012). Flavonoids are a class of

plant secondary metabolites which have been reported to play important physiological actions such as anti-oxidant, anti-atherosclerotic, anti-inflammatory, antitumor, anti-osteoporotic and antiviral activities with a insignificant side effects (Nijveldt *et.al.*, 2001). The saponins which are surface-active glycosides, have been shown immensely investigated for their immunostimulant, hypocholesterolaemic and anticarcinogenic potency (Francis *et.al.*, 2002). Mahagoni seed pod posses all the phytochemical groups in a considerable amount signifying its pharmacological value.

Carbohydrates are major energy source for the individuals, which are present in the diet in the form of polysaccharides and simple monosaccharide. Monosaccharide sugars such as glucose, fructose, galactose are directly taken up by intestinal epithelial cells (Mueckler M, 1994). In case of complex polysaccharides, they have to be digested into simpler monosaccharides prior absorption. Digestion of these complex polysaccharides takes place with the aid of enzymes (Levin, 1994). Enzymes such as  $\alpha$ -amylase,  $\alpha$ -glycosidase, sucrase and lactase play a crucial role in complete digestion of polysaccharides into simpler monosaccharide units. The liberated mono-saccharides diffuse to intestinal epithelial cells where they are taken by the passive diffusion, facilitated diffusion through transporters named Glut transporters and by co-transport with other ions, mainly sodium ion (Mueckler M, 1994). The process of glucose absorption is hindered if any of the above process is hindered. Phytochemicals/synthetic components which retard any of the processes can be considered as anti hyperglycemic agents and act by inhibiting the entry of glucose into blood stream.

Glucose adsorption onto adsorbents is a physical phenomenon, in the intestine the adsorption of glucose onto food matrix can result in decreased free glucose in the solution, thus can reduce the available glucose for uptake by the intestinal epithelial cells (Gallagher *et.al.*, 2003). The seed pod powder has shown good glucose adsorbing and glucose diffusion retardation potential thus indicating its ability to decrease the glucose availability to diffusion into blood stream. Fiber is one of the main components in the bark and seed coat of the most plants and the insoluble fiber has shown to have potent glucose adsorbing potency (Aditomre *et.al.*, 1990), thus fiber content in the seed pod powder may be component responsible for glucose adsorption and glucose retardation index. Inhibition of  $\alpha$ -amylase decreases the starch breakdown and thus breakdown of complex carbohydrate into simple absorbable sugars (William and Schenker, 1976).

### Conclusion:-

Preliminary assays in the present investigation indicated the anti-diabetic potential of Swietenia mahagoni seed pod. The observed activity may be attributed to the presence of potential bio actives in the seed pod. Further research support is needed to validate the observed potential.

### References:-

1. Aditomre, J., Eastwood, M. A., Edwards, C., & Brydon, W. G. (1990). Dietary fiber: in vitro methods that anticipate nutrition and metabolic activity in humans. *The American journal of clinical nutrition*, 52(1), 128-134.
2. Bickel, H., & Schultz, G. (1976). Biosynthesis of plastoquinone and  $\beta$ -carotene in isolated chloroplasts. *Phytochemistry*, 15(8), 1253-1255.
3. Boyle, J. R., & Ek, A. R. (1972). An evaluation of some effects of bole and branch pulpwood harvesting on site macronutrients. *Canadian Journal of Forest Research*, 2(4), 407-412.
4. Cirillo, V. P. (1962). Mechanism of glucose transport across the yeast cell membrane. *Journal of bacteriology*, 84(3), 485-491.
5. Francis, G., Kerem, Z., Makkar, H. P., & Becker, K. (2002). The biological action of saponins in animal systems: a review. *British journal of Nutrition*, 88(06), 587-605.
6. Gallagher, A. M., Flatt, P. R., Duffy, G., & Abdel-Wahab, Y. H. A. (2003). The effects of traditional antidiabetic plants on in vitro glucose diffusion. *Nutrition research*, 23(3), 413-424.
7. Gallagher, A. M., Flatt, P. R., Duffy, G., & Abdel-Wahab, Y. H. A. (2003). The effects of traditional antidiabetic plants on in vitro glucose diffusion. *Nutrition research*, 23(3), 413-424.
8. Grabley, S., & Thiericke, R. (1999). Bioactive agents from natural sources: trends in discovery and application. In *Thermal Biosensors, Bioactivity, Bioaffinity* (pp. 101-154). Springer Berlin Heidelberg.
9. Gross, G. G., Hemingway, R. W., & Yoshida, T. (Eds.). (2012). Plant polyphenols 2: chemistry, biology, pharmacology, ecology (Vol. 66). Springer Science & Business Media.



10. Hudson, B. J. F., & El-Difrawi, E. A. (1979). Sapogenins of the seeds of four Lupin species. *Journal of plant foods*.
11. Levin, R. J. (1994). Digestion and absorption of carbohydrates--from molecules and membranes to humans. *The American journal of clinical nutrition*, 59(3), 690S-698S.
12. Lucy D, Anoja S, Chu-Su Y (2002). Alternative therapies for Type 2 diabetes. *Altern. Med. Rev.* 7:45-58.
13. Miliauskas, G., Venskutonis, P. R., & Van Beek, T. A. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food chemistry*, 85(2), 231-237.
14. Mueckler, M. (1994). Facilitative glucose transporters. *European Journal of Biochemistry*, 219(3), 713-725.
15. Mueckler, M. (1994). Facilitative glucose transporters. *European Journal of Biochemistry*, 219(3), 713-725.
16. Naveen, Y. P., & Urooj, A. (2015). Phytochemical, proximate composition and antioxidant potential of *Swietenia mahagoni* leaves. *Asian J. Pharm. Res.* Vol, 5(3), 161-166.
17. Nijveldt, R. J., Van Nood, E. L. S., Van Hoorn, D. E., Boelens, P. G., Van Norren, K., & Van Leeuwen, P. A. (2001). Flavonoids: a review of probable mechanisms of action and potential applications. *The American journal of clinical nutrition*, 74(4), 418-425.
18. Ou, S., Kwok, K. C., Li, Y., & Fu, L. (2001). In vitro study of possible role of dietary fiber in lowering postprandial serum glucose. *Journal of agricultural and food chemistry*, 49(2), 1026-1029.
19. Prinz, P. N., Weitzman, E. D., Cunningham, G. R., & Karacan, I. (1983). Plasma growth hormone during sleep in young and aged men. *Journal of Gerontology*, 38(5), 519-524.
20. Shane-McWhorter, L. (2001). Biological complementary therapies: a focus on botanical products in diabetes. *Diabetes Spectrum*, 14(4), 199-208.
21. Sheela, C. G., & Augusti, K. T. (1992). Antidiabetic effects of S-allyl cysteine sulphoxide isolated from garlic *Allium sativum* Linn. *Indian journal of experimental biology*, 30(6), 523-526.
22. Slinkard, K., & Singleton, V. L. (1977). Total phenol analysis: automation and comparison with manual methods. *American Journal of Enology and Viticulture*, 28(1), 49-55.
23. Thorat, K., Patil, L., Limaye, D., & Kadam, V. (2012). In vitro Models for Antidiabetic Activity Assessment. *Intl J. of Res. in Pharma. and Biomed. Sci*, 3(2), 730-732.
24. William B Salt, I. I., & Schenker, S. (1976). Amylase-its clinical significance: a review of the literature. *Medicine*, 55(4), 269-289.
25. William B Salt, I. I., & Schenker, S. (1976). Amylase-its clinical significance: a review of the literature. *Medicine*, 55(4), 269-289.