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# Preliminary Phytochemical, Physicochemical & *In-vitro* anti-diabetic Evaluation of Different Extracts of *Dolichandrone falcate* Seem. Leaves

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## ABSTRACT

**AIM-** The aim of the present investigation is to study the Preliminary Phytochemical, Physicochemical & *In-vitro* anti-diabetic Evaluation of different extracts of *Dolichandrone falcate* Seem. **MATERIAL & METHODS-** The leaves of *Dolichandrone falcate* Seem. were collected from outfield near herbal garden of College of Pharmacy, India during the month of July that shows the green color with rough surface. The ash obtained as directed under total ash was boiled with 25 ml of HCl for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water, ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air dried drug. Extractive values of crude drug are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug. The correctly identified plant leaves is dried in shade at room temperature & after 4-5 days, it is formed in powder by mixer grinder. Defatted drug was subjected to extraction with dichloromethane, ethyl acetate, ethanol and finally by using aqueous system. Preliminary phytochemical screenings were performed for all the extracts for the presence of different phytochemicals i.e. fatty acids, steroids, terpenoids, flavonoids, phenolic compounds, alkaloids, Saponin glycosides etc. For the Inhibition of α-amylase and α–Glucosidase enzyme, different extracts (petroleum ether, dichloromethane, ethyl acetate, ethanol & aqueous extract) were dissolved in DMSO to obtain concentration of 10, 20, 40, 60, 80 and 100 µg/ml. **RESULTS-** The study on selected plant material shows that the difference of two consecutive weighing after drying for 30min. and cooling for 30min. in desiccators was found to be 0.08 gm. The Experimental result on selected plant material shows volume occupied by 1 gm of plant material was found to be 1.34 ml. The $IC_{50}$ value for acarbose drug was found to be 48.2µg/ml. Among all the extracts, petroleum ether and DCM extracts had highest activity as compared to other extracts. The $IC_{50}$ value for standard drug was found to be 18.2µg/ml. The test drug shows poor α-Glucosidase inhibitory activity compared to acarbose. **CONCLUSION-** The

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results obtained in this study have shown that various extracts shown significant antidiabetic activity. Among the various extracts, petroleum ether and DCM extracts had shown best antidiabetic activity.

**KEYWORDS**

Preliminary Phytochemical, Physicochemical, *In-vitro* anti-diabetic Evaluation, *Dolichandrone falcate* Seem. Leaves, α-amylase Enzyme, α–Glucosidase enzyme

## INTRODUCTION

Diabetes mellitus is a chronic metabolic disease as old as mankind and its incidence is considered to be high (4–5%) all over the world (Malan *et al*., 2011). The World Health Organization (WHO, 2008) estimates that more than 180 million people worldwide have diabetes. This number is likely to be more than double by 2030 (Mohan *et al*., 2007). Diabetes is a disease of disordered metabolism of carbohydrate, protein and fat which is caused by the complete or relative insufficiency of insulin secretion and /or insulin action (Ali *et al*., 2009). This leads to an increased concentration of glucose in the blood (hyperglycemia). Epidemiological studies and clinical trials strongly support that hyperglycemia is the main cause of complications related with coronary artery disease, cerebrovascular disease, renal failure, blindness, limb amputation, neurological complications and pre-mature death (Sangal, 2011). In developing countries, traditional and herbal medicine including the folk medicinal practice scatters to nearly 70 % of the population because of accessibility, affordability as well as the time tested dependability. They mainly use or dependent on herbal medicine because of the side effects of the most of the modern drugs. So the herbal medicine was selected for the present research (Tiwari and Rao, 2002).

Traditional plant-based medicines still exert great deal of importance to people living in developing countries and also serve as source to discovery of new drug candidates for a variety of diseases that threaten human health. Recently, natural herbal medicines have been widely applied for human beings.

As per the literature review, it has been observed that *Dolichandrone falcate* Seem. Leaves is listed among the various medicinal plants widely been used as a antibacterial, demulcent, bitter tonic, laxative, carminative, refrigerant, and febrifuge, diuretic, useful in chronic cystitis, gonorrhea and cadiotonic, acute-chronic inflammatory conditions and in treatment of diabetes mellitus.

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In the absence of any scientific evidence for their *In-vivo* and *In-vitro* anti-diabetic activity, an attempt is made to establish the *In-vitro* anti-diabetic activity of different extracts. So that we are able to come up with a more effective and potent bioactive extracts or phytoconstituents with less side effects in comparison with existing synthetic drugs.

## MATERIALS & METHODS

### Collection and authentication of the plant leaves

The leaves of *Dolichandrone falcate* Seem. were collected from outfield near herbal garden of College of Pharmacy, India during the month of July that shows the green color with rough surface. The plant leaves were washed thoroughly in tap water, dried in shade, finely powdered and used for successive extraction methods. Plant was identified by the Research Officer, Botany and herbarium specimen was submitted in Department of Pharmacognosy for the future reference.

### Determination of Physico chemical parameters (Sarija *et al.*, 2014)

#### *Determination of total Ash value*

Accurately weight about 3 gm of air dried drug was taken in a tarred silica crucible and incinerated by gradually increasing the temperature to make it dull red hot until free from carbon. Cooled and weighed, repeated for constant value. Then the percentage of total ash was calculated with reference to the air dried drug and calculated by using following formula.

$$\% \text{ ash content} = c\text{-}a/X \times 100$$

#### *Determination of acid insoluble Ash value*

The ash obtained as directed under total ash was boiled with 25 ml of HCl for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water, ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air dried drug.

#### *Determination of water soluble Ash value*

The total ash obtain was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water ignited for 15 minutes. The weight of insoluble matter was subtracted from the weight of total ash. The differences in weight represent the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

### Determination of Extractive values

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Extractive values of crude drug are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug.

***Determination of Alcohol Soluble Extractive Value***

5 gm. of air dried coarse powder of crude drug was macerated with 100 ml of 90% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18 hours. Thereafter, it was filter rapidly taking precaution against loss of solvent. Out of that filtrate, 25 ml of filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at $105^0$c and weighed. The percentage of ethanol soluble extractive value was calculated with reference to air dried drug.

***Determination of Water Soluble Extractive Value***

5 gm. of air dried coarse powder of crude drug was macerated with 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18 hours. Thereafter, it was filter rapidly taking precaution against loss of solvent. Out of that filtrate, 25 ml of filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at $105^0$c and weighed. The percentage of water soluble extractive value was calculated with reference to air dried drug.

**Determination of Loss on Drying**

Weight about 1.5 g of the powdered drug into a weighed flat and thin porcelain dish. Dry in the oven at $100^0$c or $105^0$c. Cool in a desiccators and watch. The loss in weight is recorded as moisture.

**Determination of Moisture Content:**

About 10g of leaves (without preliminary drying), after accurately weighing (weight to within 0.01g) was placed in a tarred evaporation dish. It was then dried at 105°C for 5 hours and weighed. Drying was continued and the root was weighed at 1 h interval until the difference between two successive weighing corresponded to not more than 0.25 percent. Constant weight was reached when two consecutive weighing after drying for 30min. and cooling for 30min. in a desiccators, did not show more than 0.01g difference.

**Determination of Swelling Index (WHO, 2000)**

Introduce the specified quantity of the plant material concerned, previously reduced to the required fineness and accurately weighed, into a 25 ml glass stoppered measuring cylinder. Add 25 ml of water and shake the mixture thoroughly every 10 minutes for 1 hour. Allow to stand for 3 hours at

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room temperature. Measure the volume in ml occupied by the plant material, including any sticky mucilage. Calculate the mean value of the individual determinations, related to 1 g of plant material.

**Successive extraction methods**

The correctly identified plant leaves is dried in shade at room temperature & after 4-5 days, it is formed in powder by mixer grinder. Powdered drug 100gm was weighed and packed in soxhlet. The drug was continuously extracted with petroleum ether for about 72 hours. Complete defatting was measured by placing a drop form the thimble on a filter paper give any oily spot. The mark was dried in air to remove traces of petroleum ether. Defatted drug was subjected to extraction with dichloromethane, ethyl acetate, ethanol and finally by using aqueous system. The % Yield of the Petroleum ether, Chloroform, Ethyl acetate, Ethanol & Aqueous extract of *Dolichandrone falcate* Seem. was calculated (Sarija *et al*., 2014).

**Phytochemical Screening**

Preliminary phytochemical screenings were performed for all the extracts for the presence of different phytochemicals i.e. fatty acids, steroids, terpenoids, flavonoids, phenolic compounds, alkaloids, Saponin glycosides etc (Sarija *et al*., 2014).

***In-vitro* Antidiabetic study of different extracts**

***Inhibition of α-amylase Enzyme***

Starch solution (0.5% w/v) was prepared in Tris hydrochloric acid buffer with 6.7 mM sodium chloride (pH 6.9) in boiling water for 5 min and pre incubated at 37°C for 5 min. Different extracts (petroleum ether, dichloromethane, ethyl acetate, ethanol & aqueous extract) were dissolved in DMSO to obtain concentration of 10, 20, 40, 60, 80 and 100 µg/ml. Then 0.2 ml of above prepared solution was added to the tubes containing starch solution. A total of 0.1 ml pancreatic amylase solution prepared in Tris HCl buffer (2 units/ ml) was added to the tube containing Different extracts and starch solution. The reaction was carried out at 37°C for 10 min. The reaction was stopped by adding 0.5 ml 50% acetic acid. The reaction mixture was centrifuged at 3000 rpm for 5 min 4°C. The absorbance of supernatant was measured at 595 nm (Melo & Gomes, 2006).

$$\text{Percentage Inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} * 100$$

***Inhibition of α–Glucosidase enzyme***

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α–Glucosidase Enzyme solution prepared in Tris buffer (pH 8) was added to the tubes containing increasing concentration of different extracts (petroleum ether, dichloromethane, ethyl acetate, ethanol & aqueous extract) in a concentration of 5, 10, 20, 40, 80 and 100 µg/ml at 37°C for 60 min. Then the reaction mixture was heated for 2 min in boiling water to stop reaction. The absorbance was measured at 540 nm. Percentage inhibition was calculated by using the following equation (Park *et al*., 2008).

$$\text{Percentage Inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} * 100$$

**Statistical analysis**

The values are expressed in mean ± SEM. The results were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's "t" test to determine the statistical significance. $p< 0.05$ was chosen as the level of significance. Statistical analysis was performed using Graph Pad Prism Software 5.0 version.

**RESULTS**

***Determination of physicochemical parameters***

**Table No. 1:** Physico-chemical parameters of *Dolichandrone falcate*

| S. No. | Determination of physicochemical parameters | Particulars |
|---|---|---|
| 1 | Total ash | 5.2 |
| 2 | Acid insoluble ash | 4.1 |
| 3 | Water soluble ash | 5.22 |
| 4 | Alcohol soluble extract value | 4.55 |
| 5 | Water soluble extract value | 6.97 |
| 6 | Loss on Drying | 5.33 |

***Determination of Moisture content***

The study on selected plant material shows that the difference of two consecutive weighing after drying for 30min. and cooling for 30min. in desiccators was found to be 0.08 gm.

***Determination of Swelling Index***

The Experimental result on selected plant material shows volume occupied by 1 gm of plant material was found to be 1.34 ml.

***Determination of % Yield of different extracts***

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**Table No. 2:** %Yield (w/w) of different extracts of *Dolichandrone falcate*

| S No. | Solvent | %Yield (w/w) |
|---|---|---|
| 1 | Petroleum ether | 4.77 |
| 2 | Dichloromethane | 6.89 |
| 3 | Ethyl Acetate | 5.34 |
| 4 | Ethanol | 8.52 |
| 5 | Aqueous | 7.12 |

**Phytochemical Screening**

Phytochemical screening of different extracts showed the presence of different phytochemical.

**Table No. 3:** Preliminary Phytochemical test for different extracts of leaf

| S. No. | Test | Petroleum ether | Dichloromethane | Ethyl acetate | Ethanol | Aqueous |
|---|---|---|---|---|---|---|
| 1. | Carbohydrate | | | | | |
| | ➢ Molish test | - | - | - | - | + |
| | ➢ Felling test | - | - | - | - | + |
| 2. | Glycosides | | | | | |
| | ➢ Bronteger test | - | - | + | - | + |
| 3. | Alkaloid | | | | | |
| | ➢ Mayer test | - | + | + | - | - |
| | ➢ Hager test | - | + | + | - | - |
| 4. | Phytosterol + Triterpinoids | | | | | |
| | ➢ Salkowaski test | + | + | - | + | - |
| 5. | Protein + Amino acid | | | | | |
| | ➢ Biuret test | - | - | - | - | - |
| | ➢ Ninhydrin test | - | - | - | - | - |
| 6. | Phenolic test | | | | | |
| | ➢ Ferric test | - | + | + | + | - |
| | ➢ Lead acetate test | - | + | + | + | - |
| 7. | Flavonoids | | | | | |
| | ➢ Alkaline test | - | - | + | + | + |
| 8. | Saponin | | | | | |
| | ➢ Foam test | - | - | - | - | + |
| 9 | Mucilage | | | | | |
| | ➢ Iodine test | - | - | - | - | + |
| | ➢ Ethanol test | - | - | - | - | + |

Note: (+) *ve* indicates positive result, whereas (–) *ve* indicates negative result

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**_In-vitro_ antidiabetic activity of different extracts**

***Inhibition of α– amylase***

Petroleum ether, dichloromethane, ethyl acetate, ethanol & aqueous extracts were used as a test drug and acarbose as reference standard were analyzed for α-amylase inhibitory activity at concentration of 10, 20, 40, 60, 80 and100 µg/ml. A dose dependent, gradual rise in inhibition of α-amylase was observed for all test drugs and Standard drug. The $IC_{50}$ value for acarbose drug was found to be 48.2µg/ml. Among all the extracts, petroleum ether and DCM extracts had highest activity as compared to other extracts.

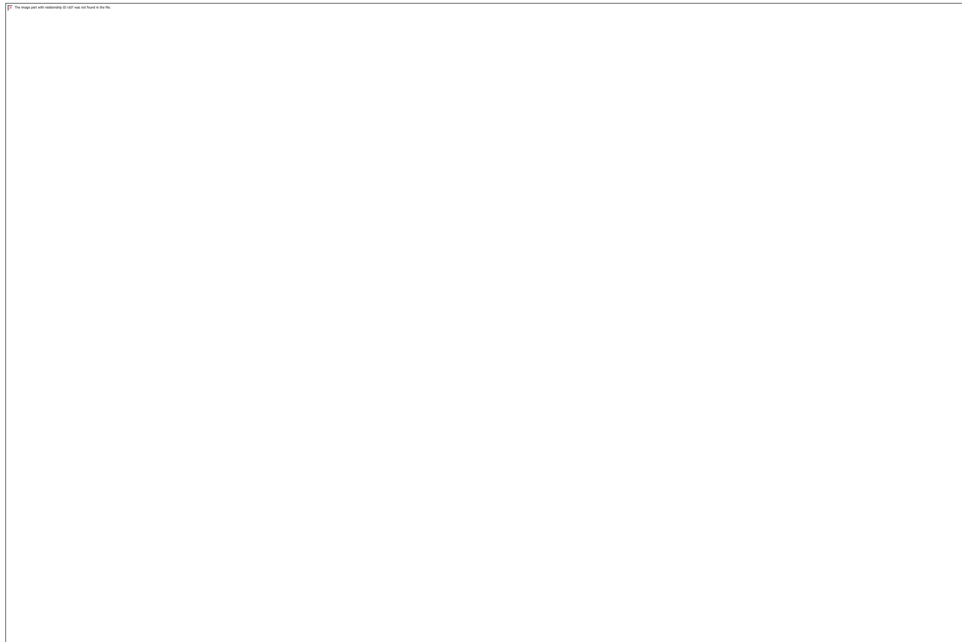

**Figure No. 1:** Effect of different extracts on Inhibition of α– amylase activity

***Inhibition of α–glucosidase***

A gradual rise in inhibitory activity of α-glucosidase was observed for Petroleum ether, dichloromethane, ethyl acetate, ethanol & aqueous extracts and acarbose as shown in figure. The concentration of all test and standard drugs varies from 5 to 100µg/ml. The $IC_{50}$ value for standard drug was found to be 18.2µg/ml. The test drug shows poor α-glucosidase inhibitory activity compared to acarbose.

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**Figure No. 2:** Effect of different extracts on α-Glucosidase inhibitory activity

**DISCUSSION**

Non-insulin dependent diabetes mellitus (NIDDM) is a multifactorial disease, which is characterized by hyperglycemia and lipoprotein abnormalities. Cell damages will in turn, result in elevated production of reactive oxygen species or ROS. High levels of ROS have been found to play a role in the pathogenesis of NIDDM (Al-Qattan *et al*., 2008). Prolonged exposure to free radicals is a pivotal cause of tissue stress and injury. The free radical permanent damage to tissue structures results from an irreversible alteration in the molecular configuration of carbohydrates, lipids, proteins and even nucleic acid bases. In diabetes, the level of free radicals was reported to increase in alloxan and streptozocin treated rats an elevated level of free radicals was detected in several tissues including the kidneys (Shabeer *et al*., 2009). Traditionally, there are various herbs are being used for the treatment of diabetes mellitus, from which merely some have been evaluated as per the modern system of medicine. From these plants only plant extracts have been prepared and evaluated for its Antihyperglycaemic activity. Most of the reported plants seem to act directly on pancreas and stimulate insulin release in the blood.

Total ash value asses the total amount of material remained after detonation and the amount of heavy metals and inorganic compounds present in the powder sample. The total ash content was 5

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times greater than acid insoluble ash, the presence of calcium oxalate crystals or acid soluble inorganic matter. The water and volatile content of a crude drug were determined by test for loss on drying. High water content will deprecate phytochemical constituents followed by hydrolysis and enhance growth of microorganisms. Hence there should be a set of confines for water content for a plant under research. Extractive values are chiefly used for the determination of exhausted or adulterated drug. The alcohol soluble extractives values were found to be higher than water soluble extractive value. Alcohol being a moderately non polar solvent, able to extract polar and non polar components yields higher extractive value.

Release of the glucose from food sources by key gastrointestinal enzymes (α-amylase and α-glucosidase) is the main factor for the postprandial rise in diabetic glucose level. α-amylase inhibitors can play an important role to reduce the rise in glucose level after meals. α- amylase is main enzyme present in pancreas responsible for the digestion of starch and absorption of glucose. Its inhibitors such as acarbose inhibit the release of glucose in the blood and thereby achieving the anti-diabetic effect (Alam *et al*., 2012).

Certainly one of the therapy approaches for diabetes is to decrease the hyperglycemia that is post-prandial delaying the utilization of glucose through the inhibition of carbohydrate-hydrolyzing enzymes, such as α-amylase and α-glucosidase (Nazreen *et al*., 2011; Karan *et al*., 2012). α-glucosidases are enzymes that increase the absorption of digested glucose from nutritional polysaccharides in the small intestinel. The α-glucosidase inhibitions of isolated compounds were indeed examined by determining the α-glucosidase inhibitory activity by the use of 4-Nitrophenyl-β-Dglucopyranosiduronic acid (pNPG) as the response substrate. The crude enzyme solution of rat's small intestine been utilized as a source of α-glucosidase, sucrose, maltase, and isomaltase. α-glucosidase catalyzes the final step of carbohydrate digestion and its particular inhibitors can delay the uptake of nutritional carbohydrates and hold back the postprandial hyperglycemia, and this can be a good activity discovery of diabetic drugs. But, it is confusing perhaps the mode of inhibition of α-amylase and α-glucosidase by isolated compounds are due to competitive and noncompetitive techniques. The theory that α-amylase and α- glucosidase showed different inhibition that is unquestionable because of structural variations pertaining to the origins of the enzymes (Lamba *et al*., 2011).

Our finding revealed that the petroleum ether and DCM extracts efficiently inhibited the enzyme as compared to the standard acarbose. α-glucosidase is responsible for the digestion of

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carbohydrates to simpler carbohydrates and its absorption in small intestine (Lamba *et al*., 2011). α-glucosidase inhibitors are known to reduce postprandial rise in diabetic glucose level (Tamil *et al*., 2010; Saha *et al*., 2008). The $IC_{50}$ value for standard drug was found to be 18.2µg/ml respectively.

The *in-vitro* antidiabetic activity revealed that petroleum ether and DCM extracts have good potential to fight against hyperglycemia, however, among these isolated compounds only α-amyrin had shown best activity in two models. Due to some ethical issues related to scarification of animal and to sacrifice minimum animal, we only proceed for *in-vivo* antidiabetic activity with petroleum ether and DCM extracts. Preliminary phytochemical screening of both extracts showed the presence of steroids, fatty acids, alkaloids, flavonoids and some traces of phenolic compounds. As per the previous literature reported by researchers all phytoconstituents have been reported for the anti-diabetic and antioxidant activity. So it might be possible that presence of these compounds may be responsible for the above mentioned activity.

## CONCLUSION

The results obtained in this study have shown that various extracts shown significant antidiabetic activity. Among the various extracts, petroleum ether and DCM extracts had shown best antidiabetic activity. Besides from the obvious therapeutic importance, these components would be useful in understanding the mechanism of diseases with higher levels of cellular and molecular level. These components could serve as lead molecules for development of prospective anti-diabetic agents. Further detailed studies are required to elucidate the exact mechanism based on molecular and genetic level responsible for anti-diabetic activity. The present findings are significant for the development of alternative, inexpensive and perhaps safer strategies for the treatment of diseases.

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