Journal of Applied Pharmaceutical Science Vol. 6 (08), pp. 102-109, August, 2016
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2016.60816
ISSN 2231-3354 

In vitro aldose reductase inhibitory potential of fractions isolated from *Potentilla fulgens* roots

Suktilang Majaw[1*], Donkupar Syiem[2]

[1]Department of Biotechnology and Bioinformatics, North-Eastern Hill University, Shillong, 793022, Meghalaya, India.
[2]Department of Biochemistry, North-Eastern Hill University, Shillong, Meghalaya, 793022, India.

ARTICLE INFO

Article history:
Received on: 03/05/2016
Revised on: 13/06/2016
Accepted on: 07/07/2016
Available online: 30/08/2016

Key words:
Aldose reductase, *Potentilla fulgens*, terpenoid/phenolic fraction, TLC F-V fraction.

ABSTRACT

The present study was investigated to identify the active fraction of *P. fulgens* with aldose reductase (AR) inhibitory potential. AR is the rate limiting step of polyol pathway implicated in the onset of chronic complications of diabetes. In this study, kidney homogenates of normoglycemic and diabetic mice were used as a source of AR enzyme preparation for *in vitro* analysis. The Terpenoid/Phenolic (TP) fraction of *P. fulgens* had the lowest IC_{50} value (0.152 mg/ml) for AR than the other fractions. TP fraction was separated using thin layer chromatography (TLC) and separated TLC fractions were tested for their AR inhibitory activity. Among the TLC fractions, F-V had the lowest IC_{50} value (0.156 mg/ml) and was characterized further using High Performance Liquid Chromatography (HPLC), Infra-Red (IR) Spectroscopy and Mass Spectroscopy (MS). F-V showed absorption maxima at λ230 nm and λ280 nm. HPLC profile of this fraction showed the presence of one prominent peak with a retention time of 1.621. IR spectra of the prominent peak indicated the presence of aromatic group which is phenolic in nature. MS of the prominent peak showed m/z ratio of 458.8. The active fraction isolated from *P. fulgens* has been shown to inhibit AR in normoglycemic and diabetic mice.

INTRODUCTION

Aldose reductase (AR) is the first and rate-limiting enzyme of the polyol pathway which converts glucose to sorbitol in the presence of NADPH as a cofactor. AR under euglycemic condition plays a minor role in glucose metabolism which accounts for approximately 3% of glucose utilization whereas under hyperglycemic condition more than 30% of glucose is metabolized through this pathway (Morrison *et al*., 1970; Yabe-Nishimura, 1998; Alexiou *et al.*, 2009).

Increased glucose flux through the polyol pathway has been associated with the pathogenesis of diabetic complications via several potential mechanisms (Steele *et al.*, 1993; Van den Enden *et al.*, 1995; Hamada and Nakamura, 2004). Therefore, inhibition of AR represents an attractive strategy for prevention of diabetic complications. Although a wide variety of compounds have been identified to inhibit the AR, however, very few of them are known to exhibit sufficient therapeutic efficacy (Pathania *et al.*, 2013). New candidate drugs have poor pharmacokinetic properties and/ or unacceptable side effects (Foppiano and Lombardo, 1997; Costantino *et al.*, 1999; Schemmel *et al.*, 2010). Hence, there is a need to develop new inhibitors against AR taking into account the efficacy, selectivity and safety issues. For diabetes and its complications, natural compounds of therapeutic value are highly sought after (Hung *et al.*, 2012). There is an increased interest in recent times to identify sources using medicinal plants for their therapeutic properties. As plant products are mostly free from adverse effects (Rao *et al.*, 2010), they are used as a source of traditional medicine in treating various ailments including diabetes mellitus. Plant extract and their derivatives like phenolics and flavonoid compounds are active inhibitors to AR enzyme (Lindstad *et al.*, 1994; Reddy *et al.*, 2011; Veeresham *et al.*, 2013).

* *Corresponding Author*
Suktilang Majaw, Department of Biotechnology and Bioinformatics, North-Eastern Hill University, Shillong, 793022, Meghalaya, India
mail id:smajaw.nehu@gmail.com / smajaw@nehu.ac.in

We have earlier evaluated *Potentilla fulgens* L. (family: Rosaceace) and found that the crude extract have the potential to inhibit AR at the enzymatic level in animal model (Syiem and Majaw, 2010). *P. fulgens*, commonly found at higher altitude of Khasi Hill, Meghalaya, India have also been reported to possess hypoglycemic, anti-hyperglycemic, anti-tumor, anti-hypolipidemic and antioxidant activity (Syiem *et al.*, 2002; Syiem *et al.*, 2003; Syiem *et al.*, 2009a; Syiem *et al.*, 2009b). In this study we have isolated active component possessing *in vitro* AR inhibitory activity from the fractions of *P. fulgens* root extract with lowest inhibitory concentration (IC_{50} value) using techniques such as TLC, HPLC, UV-Vis, IR and MS analysis.

MATERIALS AND METHODS

Chemicals and reagents

Alloxan, DL-glyceraldehyde was procured from Sigma Chemical Co. (USA), nicotinamide adenine dinucleotide phosphate (NADPH), hexane, ethylacetate, methanol, chloroform, acetonitrile, was from Sisco Research Laboratories (SRL), India. Other chemicals were of analytical grade obtained from Qualigens and SRL, India.

Experimental animal

Healthy, Swiss albino mice of approximately 6 months old were used for the study. Mice were housed in a room kept under controlled conditions with temperature maintained at 22°C on a 12h light: 12h dark cycle and were fed with balanced mice feed obtained from Pranav Agro Industries Ltd, New Delhi. All efforts were made to minimize both the number of animals used and unwanted stress or discomfort to the animals throughout the experimental procedures.

Plant material

P. fulgens L. was collected from Shillong peak area of Meghalaya and identified by Dr. P. Gurung, Department of Botany, North-Eastern Hill University, Shillong (voucher number 464). The roots were separated, weighed, washed, shredded and oven dried at 40°C. It was then powdered and utilized for preparation of plant extracts.

Preparation of plant extracts

(a) The powder was homogenized and extracted with aqueous-methanolic solution (1:4). The mixture was filtered and the filtrate was lyophilized and was used as a crude extract as per the method of Harborne (1998).

(b) Terpenoid/phenolic (TP) fraction was obtained by evaporating the filtrate (from step a) to 1/10 volume (40°C) followed by acidification with 2M H_2SO_4 to pH 0.89. This was further, extracted with chloroform (×3). The chloroform layer was separated from the aqueous layer. The chloroform layer was further evaporated to yield the TP fraction.

(c) The aqueous layer obtained in step (b) was basified to pH 10 with ammonia solution and further extracted with chloroform: methanol (3:1, twice). The chloroform-methanol layer was separated and evaporated to yield the major alkaloid (MA) fraction. Quaternary alkaloid (QA) fraction was obtained by evaporating the aqueous layer followed by extraction with methanol.

The yield percentage of each extract was calculated as per the equation given below:

$$\text{Yield Percentage (\%)} = \frac{\text{Mass of extract}}{\text{Mass of sample}} \text{ x } 100\%$$

Experimental Design

For AR inhibitory activity, varying concentration of crude extract of *P. fulgens* was tested on AR using kidney homogenate from normoglycemic mice as per the method of Guzmán and Guerrero (2005) with modification. Crude extract *of P. fulgens* was tested to compare the analysis with TP, MA and QA fractions of *P. fulgens* roots.

The crude extract and the fraction exhibiting the lowest IC_{50}value were also tested for AR inhibitory activity using kidney enzyme solution of diabetic mice in order to compare the IC_{50} value against AR in kidney of normoglycemic mice. The selected fraction was further separated using TLC and the subsequent separated TLC fractions were tested for their inhibitory effect against AR. The TLC fraction showing the maximum inhibitory activity was selected for HPLC separation and then, IR and MS analysis was performed for the isolated HPLC peak.

Preparation of diabetic mice

Animals were administered alloxan monohydrate prepared in acetate buffer (0.15 M, pH 4.5) via intraperitoneal route (Syiem *et al.*, 2002). Prior to administration, mice were fasted overnight but given water ad libitum. The blood samples collected were analyzed for glucose levels employing glucostix with the blood glucometer. Normoglycemic mice have blood glucose level in the normal range (80-120 mg/dl) and mice with more than 3-4 fold increased in blood glucose were considered diabetic.

Tissue preparation

The kidney tissue was homogenized in 2.5 volumes of cold 0.225 M sucrose-Tris buffer (pH 7.4), and centrifuged at 9000 xg for 15 min. The supernatant was further centrifuged at 16,000 xg for 30 min. The pellet was discarded and the supernatant was used as enzyme preparation for AR.

In vitro AR inhibition assay

Inhibition of AR was assayed according to Haraguchi *et al.*, 1997 with some modifications. The reaction mixture was prepared at 25ºC, in a total volume of 1 mL, containing 50 mM Na-phosphate buffer (pH 6.5), 0.2 mM NADPH, 100 µl enzyme preparation and 100 mM dl-glyceraldehyde as a substrate with or without crude extract/fractions. The reaction was initiated by

addition of NADPH and absorbance measurements were taken at λ340 nm. 1 M $NaHCO_3$ was added at the end of the 30 min incubation period. A negative control was prepared in 5% DMSO in Na-phosphate buffer (pH 6.5). The enzyme inhibition (%) was calculated using following formula:

$$\text{Inhibition\%} = \frac{\Delta \text{ Abs. (Negative Control)} - \Delta \text{ Abs. (Extract)}}{\Delta \text{ Abs. (Negative Control)}} \text{ X } 100$$

Where, Abs. is the absorbance

The experiments were run in triplicate and the concentration of extracts required to inhibit 50% (IC_{50}) of the AR activity were determined by linear regression analysis between the inhibition percentage versus the extract concentration by using the Excel program. Protein concentrations were determined according to the method of Bradford (1976) using bovine serum albumin (BSA) as the standard.

Thin layer chromatography

The TP fraction showed the lowest IC_{50} value and was further separated by TLC (Wagner and Bladt, 1996) using silica gel Grade GF-254 with $CaSO_4$ as binder using solvent system, Hexane: ethyl acetate (1:1). It was visualized using 10% ethanolic ferric chloride followed by heating the plates at 100°C. Individual fractions were separated based on their R_f values.

$$R_f = \frac{\text{Distance travelled by the sample}}{\text{Distance Travelled by the solvent}}$$

Separated fractions were re-run on the same solvent system to check for their homogeneity. Fractions showing only one spot were tested for AR inhibitory activity. TLC fraction with lowest IC_{50} value was selected for further studies.

HPLC profile of TLC fraction

The selected TLC fraction was pooled through repeated separation, dissolved in methanol and centrifuged at 3000 rpm to sediment and remove silica gel. The supernatant evaporated in an oven (below 40°C) and the obtained sample (1 mg/ml) was dissolved in 100% methanol followed by filtration through a membrane filter (pore size of 0.45 µm/13 mm).

The filtrate was scanned under UV to determine the λmax using Cary win-50 UV/Vis spectrophotometer and then applied to HPLC (Waters Pvt Ltd) using C-18 symmetry (bondapak), 3.9 × 150 mm, reverse phase column. Sample elution using isocratic mode was carried out with the mobile phase comprising acetonitrile and water (9:1), flow rate was maintained at 1 ml/min with a pressure of 800 psi and detected at λ230/280 nm using HPLC-2487 detector. The sample eluted from the HPLC column was collected using a fraction collector (WFC-44274).

IR spectra and MS analysis of HPLC fraction

The selected HPLC fraction was analyzed as a neat film between two potassium bromide (KBr) plates using FT-IR Spectrum BX (Perkin Elmer Infrared Spectrophotometer). The IR spectrum was recorded between 4000-400 wave numbers (cm^{-1}).

The major peak of the TLC fraction separated by HPLC were pooled and concentrated. The concentrated sample was dissolved in methanol and 20 µl loaded onto Waters-MS using an Xterra MS. Micromass ZQ (Multimode Ionization) from Waters was used to generate the mass spectra of the sample. Each peak corresponds to the m/z value of the fragmented sample. Thus, the peak with highest m/z value was taken as the parent molecular ion-mass.

Total polyphenolic content and flavonoid content

The total polyphenolic content of TP fraction was determined following the method of Miraliakbari and Shahidi (2008). 200 µl of TP fraction dissolved in methanol and mixed with 300 µl of 3% HCl was vortexed and allowed to stand for 3 min. 100 µl of acidified mixture was added to 1ml of 3% sodium bicarbonate followed by 20 µl of Folin-Ciocalteu reagent and allowed to stand at room temperature for 30 min. Absorbance was measured at λ760 nm. The results are calculated in mg/ml gallic acid equivalents (GAE) which is a reference standard for this method. Data were represented as mean ± standard error mean (SEM) using 5 separate experiments.

Flavonoid content of TP fraction was determined by the method of Chang *et al*., (2002). 10 mg of quercetin (standard)/TP fraction(25-100 µg/mL) dissolved in 80% ethanol. 0.5 mL of standard/TP fraction were mixed with 1.5 mL 95 % ethanol and then treated with 0.1 mL of 10 % aluminium chloride, 0.1 mL of 1M potassium acetate followed by 2.8 mL of distilled water. Absorbance was measured at λ415 nm. Data were represented as mean ± standard error mean (SEM) using 5 separate experiments.

RESULTS AND DISCUSSION

We found that the yield of crude extract (w/w from dried starting material) was 7.76% whereas the yield percentage of the TP, MA and QA fractions was 0.20 %, 0.78 % and 17.22% respectively.

AR *in vitro* inhibition assay was performed using enzyme solution of kidney tissue from normoglycemic and diabetic mice. Protein concentration of enzyme solution from normoglycemic and diabetic kidney was 8 mg/ml and 8.4 mg/ml respectively. The crude extract of *P. fulgens* roots was found to have IC_{50} value of 0.116 mg/ml and 0.133 mg/ml in kidney of normoglycemic and diabetic mice respectively (Table 1-2). The TP fraction showed IC_{50} value of 0.1522 mg/ml while MA and QA fractions exhibited IC_{50} concentration of 0.1829 mg/ml and 0.602 mg/ml respectively in kidney tissue of normoglycemic mice (Table 1).

Table 1: Inhibitory activity of crude extract, major alkaloid (MA), quaternary alkaloid (QA), terpenoid/phenolic (TP) and TLC separated fractions (F-I to F-VI) of *P. fulgens* against AR of kidney tissue from normoglycemic mice.

Sl.No.	Extracts	IC_{50} (mg/ml)
1	Crude extract	0.116
2	MA Fraction	0.1829
3	QA Fraction	0.602
4	TP Fraction	0.152
5	Fraction F-I	0. 354
6	Fraction F-II	0.737
7	Fraction F-III	0.265
8	Fraction F-IV	0.328
9	Fraction F-V	0.157
10	Fraction F-VI	0.216

Data for IC_{50} values are the average of three independent experiments.

Since, the IC_{50}value of TP fraction was lowest compared to MA and QA fractions, TP fraction was further tested for its AR inhibitory assay using kidney tissue of diabetic mice, and the IC_{50} value was found to be 0.1573 mg/ml (Table 2).

Table 2: Inhibitory activity of crude extract, terpenoid/phenolic (TP) and TLC separated fraction F-V of *P. fulgens* against AR of kidney tissue from diabetic mice.

Sl.No.	Extracts	IC_{50} (mg/ml)
1	Crude extract	0.133
2	TP Fraction	0.1573
3	Fraction F-V	0.1654

Data for IC_{50} values are the average of three independent experiments.

As observed in this study, the IC_{50} value of crude extract was found to be lowest compared to that of the TP, MA and QA fractions. This may be due to the synergistic effect of the compounds present in the crude extract. It is not always possible, however, to isolate the bioactive compounds/agents in a plant and cases are known where attempts at such isolation have resulted in loss of activity due to instability of the compounds (Harborne, 1992). It is also likely that the chemical entities degrade during the fractionation process. Many reports and reviews have highlighted similar observations that specific activities/function associated with a plant or its extract is lowered following fractionation and purification process as seen for *Zingiber officinale* (Rates, 2001) and ginseng (Hamburger and Hostettmann, 1991; Rates, 2001). The crude extract of *P. fulgens* showed lowest IC_{50} as it may contain complex mixture and inert components which are known to influence stability, bioavailability and excretion of the active component. The IC_{50} concentration for *in vitro* AR inhibitory assay showed by the crude extract and TP fraction was found to be higher in diabetic mice than in normorglycemic mice, implying lowered inhibitory efficacy. Previous studies have shown that AR isolated from diabetic or hyperglycemic tissues is less susceptible to inhibition and is kinetically different from the enzyme purified from normoglycemic or euglycemic human or animal tissues (Das and Srivastava, 1985; Srivastava *et al.*, 1985; 1986a, b; Chandra *et al.*, 2002). These changes in the inhibitor sensitivity and the kinetic properties have been reported upon thiol oxidation of purified protein *in vitro*, suggesting that diabetic changes in AR may be due to redox modification of its cysteine residues (Cappiello *et al.*, 1996). Thus, the higher IC_{50} concentration observed in this study may possibly be due to similar reason where diabetic condition results in lower susceptibility, hence, require higher concentration of extract to inhibit the enzymes of the pathway. Among the TP, MA and QA fractions, the IC_{50} concentration of TP fraction for AR was the lowest, hence, TP fraction was selected for further separation using TLC. As shown in Figure 1, six TLC fractions were observed with R_f of 0.95, 0.84, 0.75, 0.67, 0.61 and 0.48 corresponding to fraction F-I, F-II, F-III, F-IV, F-V, F-VI respectively. The separated fractions corresponding to different R_f values were further tested for their AR inhibitory activity (Table 1). The IC_{50} value of TLC fractions F-I, F-II, F-III, F-IV, FV and F-VI were found to be 0.354, 0.737, 0.265, 0.328, 0.157 and 0.216 mg/ml respectively. Since, F-V among the TLC fractions exhibited the lowest IC_{50} value, hence, TLC fraction F-V was tested for AR inhibitory activity of the kidney AR of diabetic mice where the IC_{50} value was found to be 0.1654 mg/ml as shown in Table 2.

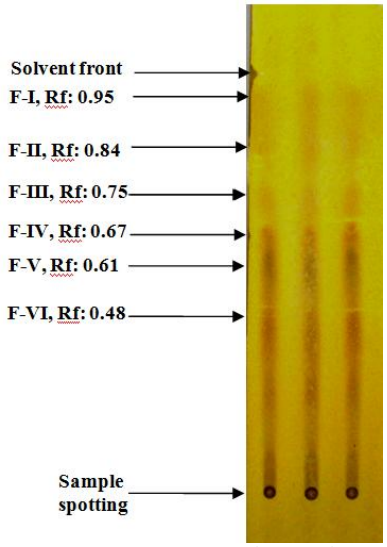


Fig. 1: Thin layer chromatography analysis of the TP fraction of *P. fulgens.* F-I to F-VI are Separated fractions based on their R_f.

TLC fraction F-V showed the lowest IC_{50} concentration compared to the other TLC fractions and hence, was selected for UV, HPLC, IR and MS analysis. Total polyphenolic and flavonoid content of TP fraction was also determined to confirm the presence of phenolics and flavonoid in the fraction.The total phenolic content of the TP fraction of *P. fulgens* was found to be 54 mg/g dry material with flavonoid content being 31 mg/g of dry material respectively (Table 3).

Table 3: Total polyphenolic and flavonoid content of the TP fraction of *P. fulgens* roots (mg/g of dry material).

Sl No.	Content	Mean ± SEM
1	Total phenolic Content (mg/g of dry material)	54 ± 0.56
2	Flavonoid content (mg/g of dry material)	31 ± 0.38

Values represented as Mean ± SEM. Mean (M) values are obtained from 5 separate experiments; SEM (±) Standard Error Mean.

The UV spectrum of TP fraction showed absorption maxima at λ205 nm, 230 nm, 250 nm and 280 nm (Figure 2a) whereas the isolated active entity (F-V) exhibited absorption maxima in the UV range at λ230 nm and 280 nm (Figure 2b).

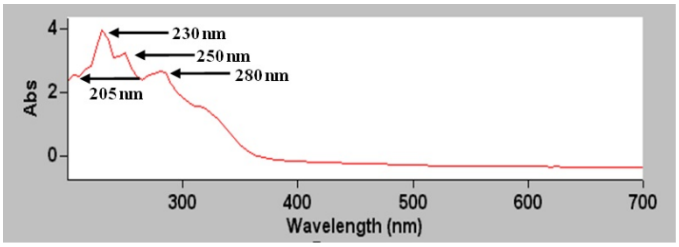


Fig. 2a: UV/Vis-spectra of TP fraction of *P. fulgens* using Cary UV-50 spectrophotometer with wavelength (λ) scan ranging from 200 to 700nm.

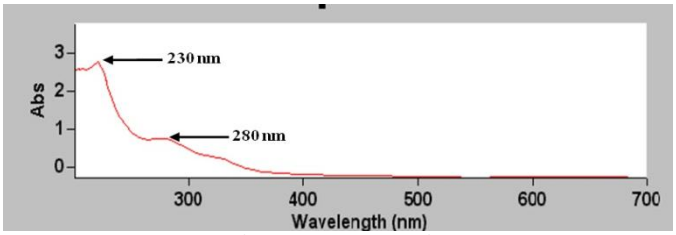


Fig. 2b: UV/Vis-spectral of the separated TLC fraction F-V using Cary UV-50spectrophotometer with wavelength (λ) scan ranging from 200 to 700nm.

HPLC chromatographic profile of TLC fraction F-V showed the presence of one prominent peak with a retention time of 1.621 and 3 minor peaks with retention time of 1.933, 2.787 and 3.449 respectively (Figure 3). This shows that the active TLC fraction (F-V) was not completely resolved by TLC. The most prominent peak in HPLC was selected for IR and MS study so as to generate a spectral finger print of this entity.

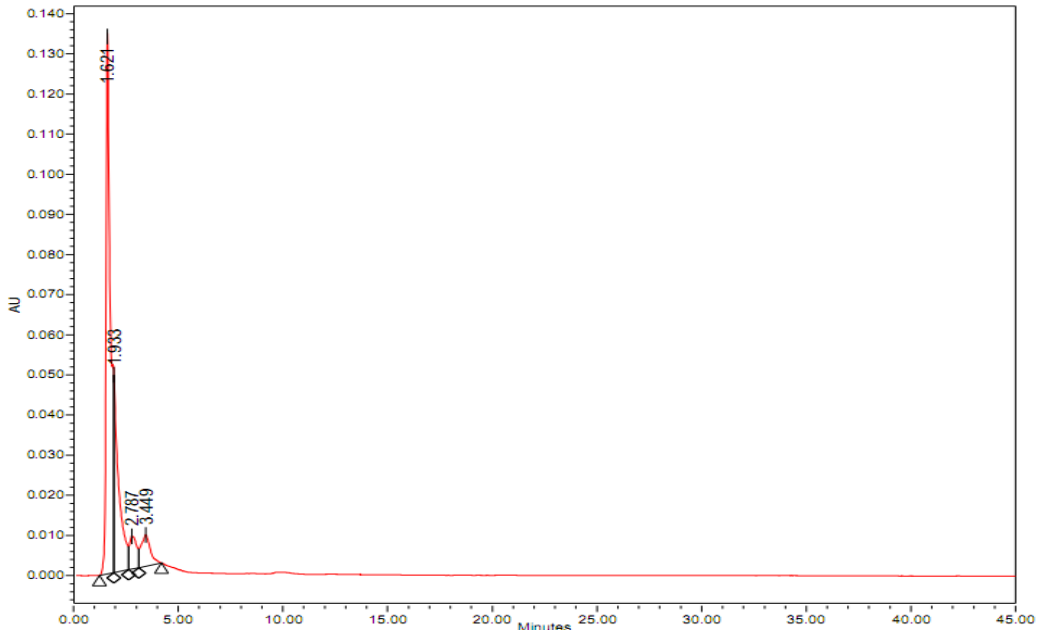
Figure 4 shows IR spectra of the major HPLC peak and the following absorption linear characteristics wave number (υ) was observed. IR (KBr) cm^{-1} of 3416, 2925, 1633, 1566, 1420, 1096 and 605, correspond to the hydroxyl groups of alcohols and phenols recognized from their typical O—H stretching absorptions in the region around 3650-3200 cm^{-1} which is left of the aliphatic C—H stretch (around 2925 cm^{-1}). The peak 1633 cm^{-1} may indicate C=O and 1566 cm-1 may be due to C=C aromatic ring (Yam *et al.*, 2010). Kudzin and Nord (1951) relate the absorption band at 1420 cm^{-1} to deformation vibrations of the CH-group in the aromatic ring. The peak 1096 cm^{-1} may be due to C-O stretching of phenols (Dick *et al.*, 2002). The signal at 605 cm^{-1} indicates –OH deformation in phenols (Ahmad *et al.*, 2010). IR spectra indicate the presence of aromatic group which is phenolic in nature.

While the mass profile of the HPLC fraction corresponding to retention time of 1.621 exhibited a m/z ratio of 454.8 (Figure 5). It may be pertinent to mention that biological entities largely follow the Lipinsky's rule (Lipinsky *et al.*, 1997). The rule was formulated by Christopher A. Lipinski in 1997, based on the observation that most medication drugs are relatively small and lipophilic molecules. The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion ("ADME"). The rule is important for drug development where a pharmacologically active lead structure is optimized step-wise for increased activity and selectivity, as well as drug-like properties as described by Lipinski's rule and one of the criteria was that the molecular weight of the active component should not be greater than 500 Da. Thus, the active component separated through HPLC possesses the qualities of active drug. These and similar studies attest to the continuing value of natural products as templates for drug design.

It may be mentioned that other studies have shown among the wide range of natural products or secondary metabolites that the phenolic compounds exhibit a wide range of biological activity in particular potential anti-diabetic properties (Brahmachari and Gorai, 2006a,b; Brahmachari, 2009). Polyphenolic are ubiquitous in the plant kingdom and are classified into three major groups: phenolic acids, flavonoids, and tannins (Wrolstad *et al.*, 2005). Phenolic acids include hydroxybenzoic, hydroxyphenylacetic, and hydroxycinnamic acids. The family of flavonoids includes mainly flavonols, flavanols, flavones, flavanones, isoflavones and anthocyanins. According to Hulse *et al*. (1980) polyphenolics are a set of phytochemicals with the molecular weight ranging from 150-30,000 Da, mainly consisting of phenolic compounds and their derivatives, flavonoids and tannins.

Flavonoids are benzo-γ-pyrone derivatives containing several hydroxyl groups attached to ring structures C_6–C_3–C_6. They include: flavonols (e.g., quercetin, kaempferol), flavones (e.g., luteolin), flavanols (e.g., catechin), isoflavones (e.g., genistein). Flavonoids are known to exhibit an inhibitory effect on AR (Haraguchi *et al.*, 1996; Kim *et al.*, 2001; Patra and Chua, 2010). Detection of the polyphenolics has been commonly based on the absorptive measurement at characteristic wavelengths. All polyphenolics absorb in the UV region (Robards and Antolovich, 1997). The result of the UV spectrum indicated that the active entity may fall in the flavanols group with absorption maxima at λ230 nm and λ280 nm.

Flavanols (sometimes referred to as Flavan-3-ols) are a class of flavonoids that use the 2-phenyl-3,4-dihydro-2H-chromen-3-ol skeleton. Catechin and epicatechin with epigallocatechin and gallocatechin are some of the most common examples of flavanols. Other species of Potentilla like *P. alba* have been found to contain catechin (Gritsenko and Smik, 1977); epicatechin has been detected in *P. erecta* (Vennat *et al.*, 1994), *P. anserine* hasepigallocatechin (Kombal and Glasl, 1995). Thus, *P. fulgens* also have high possibility of possessing these flavonols group, thus, further studies are required for confirmation.



Sl No.	Retention time (min)	Area	% Area	Height	Peak Type
1	1.621	1989982	62.47	134663	Unknown
2	1.933	756485	23.75	49255	Unknown
3	3.449	245244	7.70	7841	Unknown
4	2.787	193915	6.09	8199	Unknown

Fig. 3: HPLC profile of the separated TLC fraction (F-V) using C-18 reverse phase column (3.9 x 150 mm). Solvent used: Acetonitrile and water in the ratio of 9:1. Detection: at λ 230/280.

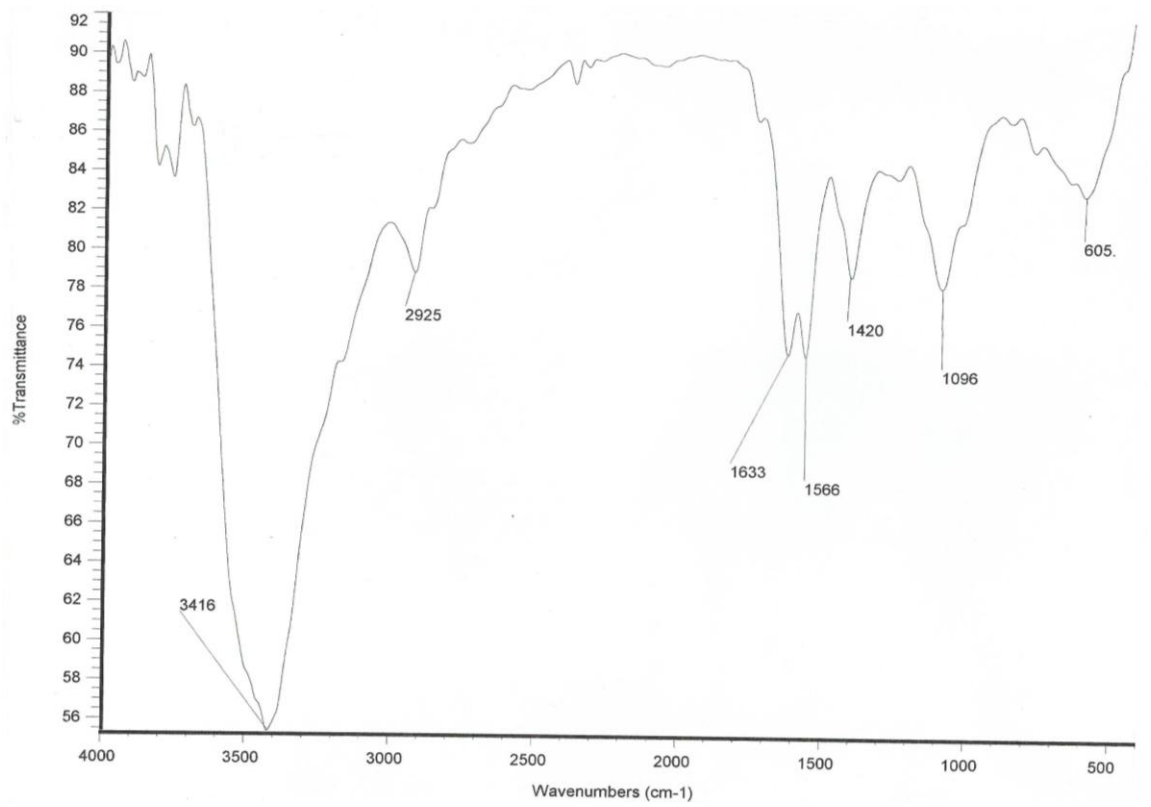


Fig. 4: IR spectra of the major HPLC peak using Perkin Elmer Infrared Spectrophotometer.

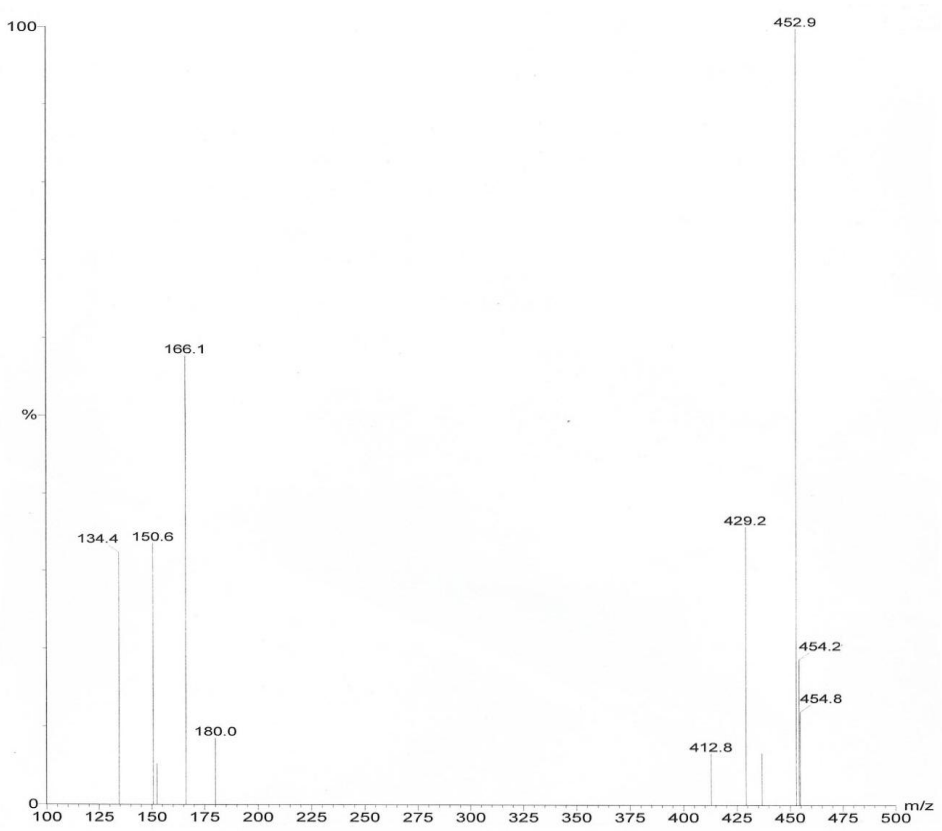


Fig. 5: Mass Spectra of the major peak of HPLC fraction showing m/z ratio of 454.8.

CONCLUSION

This study was performed to isolate and identify the active fraction possessing AR inhibitory activity from the TP fraction of *P. fulgens* roots. From the biochemical analysis, it confirms that the TP fraction contained polyphenolic and flavonoid group which was separated into different fractions by TLC where fraction F-V was found to have better inhibitory activity against AR compared to the other TLC fractions. The active fraction isolated from *P. fulgens* could be a promising anti-diabetic drug as it has the ability to inhibit the aldose reductase of the polyol pathway which is one of the mechanisms leading to diabetic complication.

ACKNOWLEDGEMENT

The authors acknowledge the Department of Chemistry, NEHU, Shillong for IR spectra and Sophisticated Analytical Instrumental Facility Centre (SAIF), NEHU, Shillong for MS analysis. The authors would like to thanks Mr. J.M. Sohtun for his assistance with the HPLC analysis.

REFERENCES

Ahmad F, Ali M, Alam P. New phytoconstituents from the stem bark of *Tinospora cordifolia* Miers. Nat Prod Res, 2010; 24:926-934.

Alexiou P, Pegklidou K, Chatzopoulou M, Nicolaou I, Demopoulos VJ. Aldose reductase enzyme and its implication to major health problems of the 21(st) century. Curr Med Chem, 2009; 16:734-752.

Bradford M.A rapid and sensitive method for the quantitation of mcrogram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem, 1976; 72:248-254.

Brahmachari G, Gorai D. Progress in the research on naturally occurring flavones and flavonols: An overview. Curr Org Chem, 2006a; 10:873-898.

Brahmachari G, Gorai D. 2006b. Progress in the research of natural flavonoids: An overview. In: Brahmachari G, ed. Chemistry of Natural Products: Recent Trends and Developments. Trivandrum: Research Signpost. 78-168.

Brahmachari G. 2009. Mother nature: An inexhaustible source of drugs and lead molecules. In: Brahmachari G, ed. Chemistry, Biochemistry and Pharmacology. New Delhi: Narosa Publishing House Pvt. Ltd. 1-20.

Cappiello M, Voltarelli M, Cecconi I, Vilardo PG, Dal Monte M, Marini I, DelCorso A, Wilson DK, Quiocho FA, Petrash M, Mura U. Specifically targeted modification of human aldose reductase by physiological disulfides. J BiolChem, 1996; 271:33539-33544.

Chandra D, Jackson EB, Ramana KV, Kelley R, Srivastava SK, Bhatnagar A. Nitric oxide prevents aldose reductase activation and sorbitol accumulation during diabetes. Diabetes, 2002; 51:3095-3101.

Chang CC, Yang MH, WEN HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal, 2002; 10:178-182.

Costantino L, Rastelli G, Vianello P, Cignarella G, Barlocco D. Diabetes complications and their potential prevention: Aldose reductase inhibition and other approaches. Med Res Rev, 1999; 19:3-23.

Das B, Srivastava SK. Activation of aldose reductase from human tissues. Diabetes, 1985; 34:1145-1151.

Dick DP, Mangrich AS, Menezes SMC, Pereira BF. Chemical and spectroscopical characterization of humic acids from two South Brazillian coals of different ranks. J Braz Chem Sci, 2002; 13(2):177-182.

Foppiano M, Lombardo G. Worldwide pharmacovigilance system and tolrestat withdrawal. Lancet, 1997; 349: 399-400.

Guzman A, Guerrero RO. Inhibition of aldose reductase by herbs extracts and natural substances and their role in prevention of cataracts. Rev Cubana Planta Med, 2005; 10:3-4.

Gritsenko OM, Smik GK. Phytochemical study of *Potentilla alba*. FarmatsevtichniiZhurnal (Kiev), 1977; 1:88-89.

Hamada Y, Nakamura J. Clinical potential of aldose reductase inhibitors in diabetic neuropathy. Treat Endocrinol, 2004; 3:245-255.

Hamburger M, Hostettmann K. Bioactivity in plants: the link between phytochemistry and medicine. Phytochemistry, 1991; 30(12):3864-3874.

Haraguchi H, Ohmi I, Sakai S, Fukuda A, Toihara Y,Fujimoto T, Okamura N, Yagi A. Effect of *Polygonum hydropiper* sulfated flavonoids on lens aldose reductase and related enzymes. J Nat Prod, 1996; 59:443-445.

Haraguchi H, Ohmi I, Fukuda A, Tamura Y, Mizutani K, Tanaka O, Chou WH. Inhibition of aldose reductase and sorbitol accumulation by astilbin and taxifolin dihydroflavonols in *Engelhardtia chrysolepis*. Biosci Biotechnol Biochem, 1997; 61:651-654.

Harborne JB. 1992. Chemicals as defence agents. In: Harborne JB, ed. Introduction to ecological Bochemistry. New York: Academic Pres. Harcourt Brace and Co publishers. 131-158.

Harborne JB. 1998. Phytochemical methods, 3rd edition, London and New York: Chapman and Hall.

Hulse JH, Laing EM, Perason, OE. 1980. Sorghum and the millets: their composition and nutritive value. London: Academic Press.

Hung HY, Qian K, Morris Natschke SL, Hsu CS, Lee KH. Recent discovery of plant-derived anti-diabetic natural products. Nat Prod Rep, 2012; 29:580-606.

Kim MK, Kim SY, Lee HS.Rat lens aldose reductase inhibitory activities of oriental medicinal plants. Agric Chem Biotechnol, 2001; 45:84-88.

Kombal R, Glasl H. Flavan-3-ols and flavonoids from *Potentilla aserina*. Planta Med, 1995; 61(5):484-485.

Kudzin SF, Nord FF. Investigations on lignin and lignifications. IV. Studies on hardwood lignin. J Am Chem Soc, 1951; 73(2):690-693.

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev, 1997; 23(1-3):3-25.

Lindstad RI, Hermansen LF, McKinley-McKee JS.Inhibition and activation studies on sheep liver sorbitol dehydrogenase. Eur J Biochem, 1994; 221:847-854.

Miraliakbari H, Shahidi F. Oxidative stability of tree nut oils. Food Chem, 2008; 111(2):421-427.

Morrison AD, Clements RS, Travis SB, Oski F, Winegrad AI.Glucose utilization by the polyol pathway in human erythrocytes. Biochem Biophys Res Commun, 1970; 40:199-205.

Pathania S, Randhawa V, Bagler G. Prospecting for novel plant-derived molecules of *Rauvolfia serpentina* as inhibitors of aldose reductase, a potent drug target for diabetes and its complications. PLoS ONE, 2013; 8(4): e61327. doi:10.1371/journal.pone.0061327.

Patra JC, Chua BH. Artificial neutral network-based drug design for diabetes mellitus using flavonoids. J Comput Chem, 2010; 32:555-567.

Rao UM, Sreenivasulu M, Chengaiah B, Jaganmohan Reddy K, Chetty MC. Herbal medicines for diabetes mellitus: a review. Int J Pharm Tech Res, 2010; 2:1883-1892.

Rates SML. Plants as source of drugs. Toxicon, 2001; 39:603-613.

Reddy GB, Muthenna P, Akileshwari C, Saraswat M, Petrash JM. Inhibition of aldose reductase and sorbitol accumulation by dietary rutin. Curr Sci, 2011; 101(9):1191-1197.

Robards K, Antolovich M. Analytical chemistry of fruit bioflavonoids. A review. Analyst, 1997; 122:11R-34R.

Schemmel K, Padiyara R, D'Souza J. Aldose reductase inhibitors in the treatment of diabetic peripheral neuropathy: a review. J Diabetes Complications, 2010; 24:354-360.

Srivastava SK, Hair GA, Das B. Activated and unactivated forms of human erythrocyte aldose reductase. Proc Natl Acad Sci USA, 1985; 82:7222-7226.

Srivastava SK, Ansari NH, Hair GA, Awasthi S, Das B. Activation of human erythrocyte, brain, aorta, muscle, and ocular tissue aldose reductase. Metabolism,1986a; 35(Suppl 1):114-118.

Srivastava SK, Ansari NH, Hair GA, Jaspan J, Rao MB, Das B. Hyperglycemia-induced activation of human erythrocyte aldose reductase and alterations in kinetic properties. Biochim Biophys Acta, 1986b; 870: 302-311.

Steele JW, Faulds D, Goa KL. Epalrestat. A review of its pharmacology, and therapeutic potential in late-onset complications of diabetes mellitus. Drugs Aging, 1993; 3:532-555.

Syiem D, Gareth S, Khup PZ, Khongwir BS, Kharbuli B, Kayang H. Hypoglycemic effect of *Potentilla fulgens* L. In normal and alloxan-induced diabetic mice. J Ethnopharmcol, 2002; 83:55-56.

Syiem D, Syngai C, Kharbuli B, Kayang H, Khongwir BS. Anti-tumor activity of crude root extract of *Potentilla fulgens*. Indian Drugs, 2003; 40:124-125.

Syiem D, Khup PZ, Syiem AB. Effects of *Potentilla fulgens* Linn. on carbohydrate and lipid profiles in diabetic mice. Pharmacologyonline, 2009a; 2:787-795.

Syiem D, Sharma R, Saio V. *In vitro* study of the antioxidant potential of some traditionally used medicinal plants of North-East India and assessment of their total phenolic content. Pharmacologyonline, 2009b; 3:952-965.

Syiem D, Majaw S. Effect of *Potentilla fulgens* L. aldose reductase activity of normal and diabetic mice. Inventi Rapid: Ethnopharmacology, 2010; 1(1):ep13.

Van den Enden MK, Nyengaard JR, Ostrow E, Burgan JH, Williamson JR. Elevated glucose levels increase retinal glycolysis and Van den Enden MK, Nyengaard JR, Ostrow E, Burgan JH, Williamson JR. Elevated glucose levels increase retinal glycolysis and sorbitol pathway metabolism. Implications for diabetic retinopathy. Invest Ophthalmol Vis Sci, 1995; 36:1675-1685.

Veeresham C, Rama Rao A, Asres K. Aldose reductase inhibitors from plant origin. Phytother Res, 2013; 28(3):317-333.

Vennat B, Bos MA, Pourrat A, Bastide P. Procyanidins from tormentil: fractionation and study of the anti-radical activity towards superoxide anion. Biol Pharm Bull, 1994; 17:1613-1615.

Wagner H, Bladt S. 1996. Plant Drug Analysis: A Thin Layer Chromatography Atlas. 2nd edition. Heidelberg, NY, USA: Springer.

Wrolstad RE, Acree TE, Decker EA, Penner MH, Reid DS, Schwartz SJ, Shoemaker CF, Smith DM, Sporns P. 2005. Handbook of food analytical chemistry: pigments, colorants, flavors, texture and bioactive food components. Hoboken, New Jersey: John Wiley & Sons Inc. 489.

Yabe-Nishimura C. Aldose reductase in glucose toxicity: a potential target for the prevention of diabetic complications. Pharmacol Rev, 1998: 50:21-33.

Yam MF, Lim V, Salma IM, Ameer OZ, Ang LF, Rosadah N, Abdulkarm MF, Abdullah GZ. HPLC and Anti-inflammatory studies of the flavonoid rich chloroform extract fraction of *Orthosiphon stamineus* leaves. Molecules, 2010; 15:4452-4466.

How to cite this article:

Suktilang Majaw, Donkupar Syiem. *In vitro* aldose reductase inhibitory potential of fractions isolated from Potentilla fulgens roots. J App Pharm Sci, 2016; 6 (08): 102-109.