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Phytochemical, enzymatic and biological studies of root extracts of *Farsetia hamiltonii* Royle

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Abstract: *Farsetia hamiltonii* Royle is a medicinal plant of Cholistan desert, Pakistan, traditionally used for treating diabetes, oxidative stress, arthritis, fever, gastrointestinal and respiratory diseases. This study represents unprecedented phytochemical, enzymatic and biological properties of *F. hamiltonii* root extracts to prove floric uses. Evaluation of Phytochemical constituents was done by screening, total flavonoid, phenolic contents and gas chromatography-mass spectrometry. The phytochemical screening revealed the presence of glycosides, bounded anthraquinones, flavonoids, saponins, steroids, coumarins and diterpenes in root extracts. Eight compounds were identified in dichloromethane extract, whereas one compound was identified in methanol extract of root part of *F. hamiltonii.* The dichloromethane extract possesses significant lipoxygenase, chymotripsin, and cholinesterase enzyme inhibition activities, whereas methanol extract possess lipoxygenase, alpha glucosidase, chymotrypsin and acetylcholinesterase enzyme inhibition activities. The antibacterial activity of methanol extract was significant against selected five microbial strains. Nine compounds were reported in root part of *F. hamiltonii* first time. The enzyme inhibition assays on anti-cholinesterase, anti-alpha glucosidase, antilipoxygenase, antichymotripsin and antibacterial activities were found significant for the extracts of root parts of *F. hamiltonii*. Therefore, results of this study justify folkloric therapeutic potential of *F. hamiltonii* root in treating diabetes, inflammations and infectious diseases.

Keywords: Alpha-glucosidase, antioxidant, *Farsetia hamiltonii* Royle, gas chromatography-mass spectrometry.

INTRODUCTION

Cholistan is a very rich land bestowed with many medicinal plants. Cholistan desert is a part of Great Indian Desert having the area 26,300 km^2. It is situated within South of Bahawalpur in Punjab reaching the Nara and Thar deserts of Sindh between 27°42' and 29°45' N latitude and 69°52' and 75°24' E longitude. The Cholistan desert have an altitude of about 112 meter above sea level (Akhter *et al.,* 2006; Ali *et al.*, 2009; Ahmed *et al.,* 2014). In the winters, the temperature of Cholistan desert touches to the freezing point with frost formation, while the summers are hot and dry with temperature rising to 51^{0}C.The flora of Cholistan desert consists of about 38 families, 106 genera and 154 species (Waris *et al.,* 2013).

Farsetia hamiltonii Royle (fig. 1) belongs to family Brassicaceae is a traditional medicinal plant of Cholistan desert and its flowering period is from March to September (Nasir and Ali, 1972). The medicinal use of plant is as antirheumatic and antidiabetic herb preparation. This plant can also be used as a cooling agent and tonic. The boiled juice of plant is applied to treat camel wound in Cholistan (Arshad *et al*., 2002). The local names are "Fareed Booti" (Bhandari, 1995) and "Gawala takri" (Raza *et al.,* 2014). According to comprehensive literature survey, no pharmaceutical and biological work has been reported on the *F. hamiltonii* roots. Therefore, our group has started phytomedicinal in depth research on previously unexplored, *F. hamiltonii* (Hayat *et al.,* 2014, 2015, 2019). In view of diverse traditional uses of the plant, the present study is designed to evaluate the crude extracts of the roots of *F. hamiltonii* for pharmaceutical constituents, enzyme inhibition and biological activities scientifically to confirm the folk uses.

MATERIALS AND METHODS

Chemicals and instruments used in study

Alpha glucosidase (EC3.2.1.20), Lipoxygenase (EC1.13.11.12), acetylcholinesterase (EC3.1.1.7), butyrylcholinesterase (EC3.1.1.8), DPPH, quercetin, acarbose, baicalein, eserine, gallic acid, dichloromethane (DCM) and methanol (MeOH) were purchased from Sigma-Aldrich, Merck and Fluka companies. Sodium hydroxide, Na_2CO_3, NaNO, $AlCl_3$, sucrose, H_2O_2, starch, KH_2PO_4, Tris-HCl buffer, DTNB [5,5-dithiobis (2-nitrobenzoic acid)], DMSO and folin ciocalteu reagent were of analytical grade and purchased from Scharlau.

The instruments used in this study were Synergy HT BioTek 96 microplate reader, Rotary evaporator (Buchi, USA), Vacuum pump, Spectrophotometer (Model Halo DB-20S, Dynamica, Australia), Gas chromatography-

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mass spectrometry (GC Agilent system, USA) and EZ-Fit Enzyme Kinetics software (Perrella Scientific Inc. Amherst, USA).

Plant collection and extraction

The fresh plant of *F. hamiltonii* was collected in March-April, 2013 from the Cholistan desert of Bahawalpur division, Punjab Province, Pakistan (fig. 2). The plant specimen was identified by research officer of Cholistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur, Pakistan. The plant specimen deposited in the same institute and specimen voucher number 3470/CIDS/IUB was allocated (fig. 3).

Fig. 1: Aerial view of *Farsetia hamiltonii* Royle.

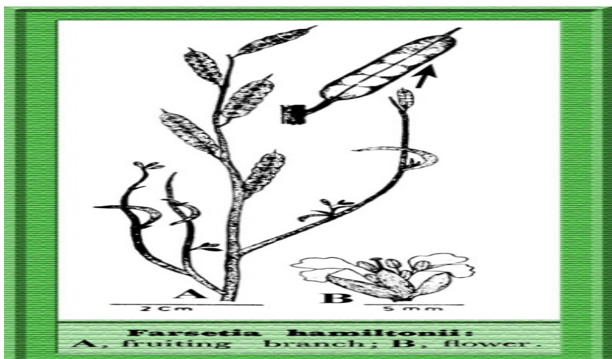

Fig. 2: Morphological characters and features of *Farsetia hamiltonii* Royle.

The roots of plant were fully cleaned from dust, shade dried for 15 days and ground in fine powder by small-scale mill. The powdered material (200g) of root part was macerated in dichloromethane for 24 hours; the process was repeated for three days. Likewise, the plant material was macerated in methanol for three days. After filtration, dichloromethane and methanol extracts were evaporated on rotary evaporator under reduced pressure to thick semi solid mass. The crude extracts were abbreviated as FHRD for dichloromethane extract of roots of *F. hamiltonii* and FHRM for methanol extract of root parts of *F. hamiltonii*. The yields of extraction of FHRD and FHRM extracts were 1.0% and 1.25%, respectively.

Phytochemical screening

The dichloromethane and methanol extracts of roots of *F. hamiltonii* were evaluated for different secondary metabolites like alkaloids, glycosides, anthraquinones, flavonoids, saponins, steroids, leucoanthocyanins, coumarins and diterpenes through standard preliminary tests. To identify the chemical groups in plant, standard procedures are followed (Brain, 1975; Trease and Evans, 1989). The glycosides, bounded anthraquinones, flavonoids, saponins, steroids, coumarins and diterpenes were present in *F. hamiltonii* root.

Fig. 3: Herberium sheet of *Farsetia himaltonii* Royle (specimen voucher No.3470/CIDS/IUB).

Determination of total flavonoid contents

Total flavonoid contents (TFC) in crude extracts of roots of *F. hamiltonii* were determined by the method reported in the literature (Zengin *et al.,* 2010). Deionized water (80µL) was mixed with 20µL plant extract (0.5mg/mL) in microplate. 5% NaNO solution (16µL) was added after that added 10% $AlCl_3$ solution (16µL) and finally 4% NaOH solution (68µL) was added. After incubation for 20min at room temperature, absorbance was determined at 510 nm. The same procedure was repeated for the standard solution of quercetin and the calibration line was constructed. The concentration of flavonoids was measured (mg/mL) on the calibration line; the TFC in extracts was expressed in terms of quercetin equivalent (mg of QE/g of extract) in table 1.

Table 1: Total flavonoids and total phenolic contents of root extract of *F. hamiltonii*.

Sample code	Flavonoids (mg QE/g of extract)	Phenolics (mg GA/g of extract)
FHR-D	60.69±0.77	21.84±0.61
FHR-M	50.34±0.49	---
Quercetin	980.42±3.11	------
Gallic acid	------	868.07±1.34

Determination of total phenol content

Total phenol content (TPC) in extracts of roots of *F. hamiltonii* was determined by folin-ciocalteu reagent (FCR) according to the method (Ismail *et al.,* 2004). The 20µL extract solution (0.5mg/mL) was mixed with FCR (90µL, 1:10 diluted with distilled water) in microplate reader and aqueous Na_2CO_3 (90µl, 6%w/v) added. The

TPC were determined at 725 nm. The same procedure was repeated for the standard solution of gallic acid and the calibration line was constructed. Based on the measured absorbance, the concentration of TPC was determined (mg/mL) from the calibration line and the TPC in extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract) in table 1.

Gas chromatography-mass spectrometry (GC-MS)

GC-MS analysis included in this study to investigate the pharmaceutical constituents of the selected medicinal plant *F. hamiltonii*. GC-MS analysis was performed (David *et al.,* 2011; Hites and Ronald, 2016) on GC Agilent system (B 7890) with mass spectrometer detector (MSD-5977A) employing the following condition: Column HP-5MS, size 30 m×0.25 mm, 0.25μ, composed of 100% dimethyl poly siloxane. The source temperature for ionization was set at 250°C. The 2μL of DCM and methanol extracts of *F. hamiltonii* (root part) were used in GC-MS analysis. The GC-MS chromatograms (fig. 4 and 5) results revealed the presence of nine (09) compounds in two different extracts of root of *F. hamiltonii*. The name, molecular formula, molecular weight and structure of the compounds are given in table 2 and 3.

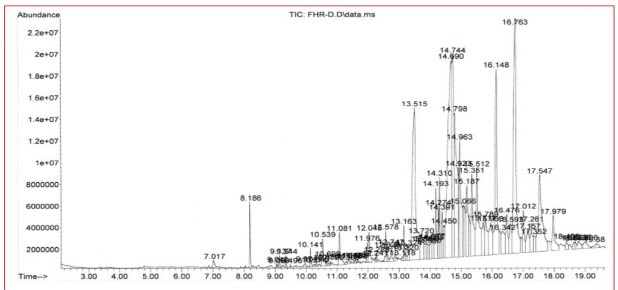

Fig. 4: GC-MS chromatogram of dichloromethane extract of root part of *F. hamiltonii*.

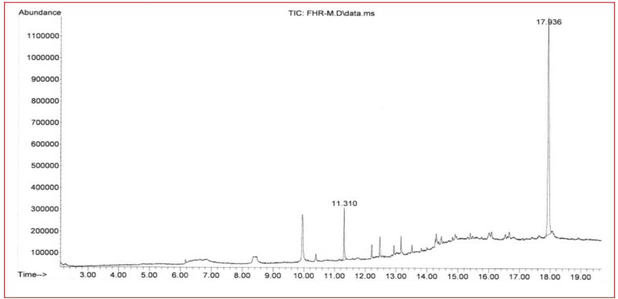

Fig. 5: GC-MS chromatogram of methanol extract of root part of *F. hamiltonii*.

Enzyme inhibition assays

Alpha glucosidase inhibition assay

The α-glucosidase inhibition assay was performed according to the slightly modified method (Pierre *et al.,* 1978). Total volume of the reaction mixture was 100μL contained 70μL phosphate buffer (50mM and pH 6.8), 10μl of test compound dissolved in methanol, followed by the addition of 10μL enzyme (0.057). The contents were mixed, pre-incubated for 10min at 37ºC and pre-read at 400nm. The reaction was initiated by the addition of 10μL of 0.5mM/well substrate. Acarbose was used as standard at 0.5mM concentration. The test sample was incubated for 30 min at 37ºC and then absorbance was measured at 400 nm (table 4).

Lipoxygenase inhibition assay

Lipoxygenases (LOXs) inhibition was assayed according to the method described in literature (Tappel, 1953). A total volume of 200μL contained 140μL KH_2PO_4 buffer (100mM, pH 8.0), 20μL plant extract (0.5μg/mL) and 15μL purified LOX enzyme (600 units/well). The contents were mixed and pre-read at 234 nm and pre-incubated for 10min at 25°C. The reaction was initiated by the addition of 25μl linoleic acid solution. The change in absorbance was observed after 10min at 234nm. Baicalein (0.5mM/well) was used as standard. The results are given in table 4.

Chymotrypsin inhibition assay

A standard procedure was followed with slight modification (Atta *et al*., 2001). The 60μL Tris-HCl buffer (50mM, pH 7.6), 10μL extract solution (0.5mg/mL) and 15μL purified α-chymotrypsin enzyme (0.9units) were mixed in 96-microwell plate. The contents were pre-read. The reaction was initiated by the addition of 15μl *N*-succinyl phenyl-alanine-*p*-nitroanilide (1.3mM). The absorbance was measured at 410nm. Chymostatin (0.5mM) was used as standard (table 4).

Cholinesterase inhibition assay

Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) inhibition assays were performed according to the method (Ellman *et al.,* 1961). In both assays, total volume of the reaction mixture 100μL contained 60μL KH_2PO_4 buffer (100mM, pH7.7). 10μL plant extract (0.5μg/mL) was added followed by the addition of 10μL enzyme. The contents were mixed, pre-incubated for 10min at 37ºC and pre-read at 405 nm. The contents were then pre-incubated for 10min at 37ºC. The reaction was initiated by the addition of 10μL of DTNB (0.5mM per well) and absorbance was measured at 405nm after 15min. Eserine was used as standard and results are given in table 4.

Biological Activities

Antioxidant activity

DPPH (1,1-Diphenyl-2-picrylhydrazyl) assay is a very simple and highly sensitive method that is based on the principle that a hydrogen donor is an antioxidant. DPPH• is one of the few stable and commercially available organic nitrogen radicals. It shows a strong absorption maximum at 517nm. The antioxidant effect of sample is directly related to the disappearance of DPPH• in test samples. The color turns from purple to yellow due to the formation of DPPH upon absorption of hydrogen from an antioxidant (Vina *et al.,* 2012). This assay was carried out

according to method ((Ellman *et al.,* 1961; Vina *et al.,* 2012). 10 µL of test solution was added in 96-wells plate followed by the addition of 90µL of 100µM methanol DPPH solution in a total volume of 100µL. The contents were mixed and incubated at 37ºC for 30min. The reduction in the absorbance was measured at 517nm using 96 well microplate reader. Quercetin was used as standard antioxidant. The results are given in table 4.

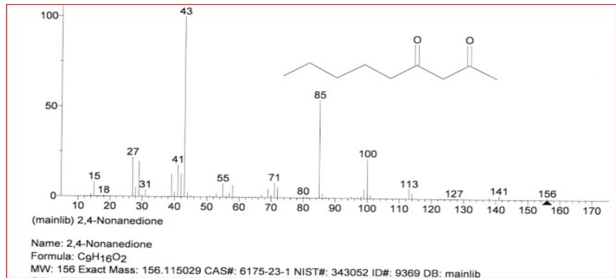

Fig. 6: Mass spectra and structure of 2,4-nonanedione.

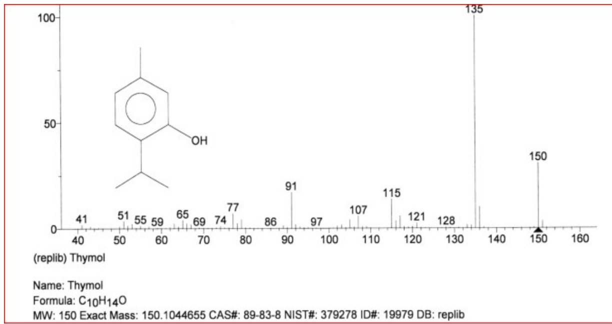

Fig.7: Mass spectra and structure of Thymol.

Antibacterial activity

The antibacterial activity was performed in sterile 96-wells microplates under aseptic environments. The method is based on the principle that microbial cell number increases as the microbial growth occurs in a log phase of growth, which results in increased absorbance of broth medium (Lee *et al.,* 2003). Two gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus*) and three gram-negative (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi*) were included in the study. The organisms were maintained on stock culture agar medium. The test sample with suitable solvents and dilutions was pipetted into wells (20µg/well). Overnight maintained fresh bacterial culture after suitable dilution with fresh nutrient broth was poured into wells (180µL). The initial absorbance of the culture was strictly maintained between 0.12-0.19 at 540nm. The total volume in each well-kept to 200µL. The incubation was done at 37°C for 16-24 hours with lid on the micro plate. The absorbance was measured at 540nm using micro plate reader, before and after incubation and the difference was noted as an index of bacterial growth. The percent inhibition calculated using the formula: Inhibition (%)=100×(X-Y)/X, where X is absorbance in control with bacterial culture and Y is absorbance in test sample. Ciprofloxacin was used as standard and minimum inhibitory concentrations (MIC_{50}) were calculated with further dilutions using EZ-Fit Enzyme Kinetics Software. Results (n=3, ± SEM) are mean of triplicate (table 5).

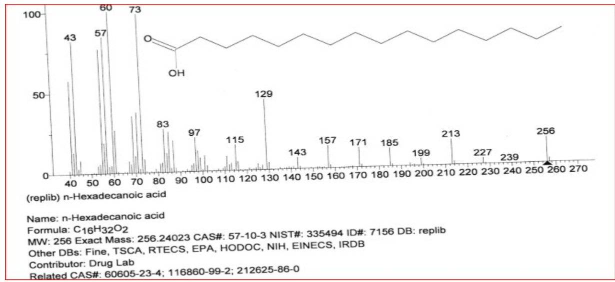

Fig. 8: Mass spectra and structure of n-hexadecanoic acid.

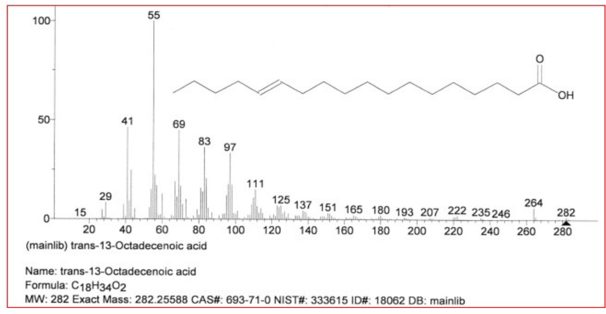

Fig. 9: Mass spectra and structure of trans-13-octadecenoic acid.

STATISTICAL ANALYSIS

The percentage enzyme inhibitions were calculated by the following formula:
Inhibition (%) =100-(Absorbance of test sample/ Absorbance of control) ×100.
IC_{50} values (concentration at which there is 50% in enzyme catalyzed reaction) compounds were calculated using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA). For the determination of IC_{50} values, test solutions were assayed at various dilutions i.e. 0.5, 0.25, 0.125, 0.0625mg/mL. All the results (table 3-6) are reported as mean±SEM (standard error of mean) of triplicate values (n=3).

RESULTS

The DCM and methanol extracts of root of *F. hamiltonii* were evaluated for secondary metabolites (Brain, 1975; Trease *et al.,* 1989) and confirmed the presence of glycosides, bounded anthraquinones, flavonoids, saponins, steroids, coumarins and diterpenes. Flavonoids totalcontents (TFC) were determined as quercetin (QE) equivalents by standard curve equation y=0.4918x+0.1604 (r^2=0.997) and expressed in mg of QE/g of extract. TFC in root extracts of dichloromethane and methanol of plant were 60.69±0.77 and 50.34±0.49, respectively (Zengin *et al.,* 2010). Total phenol contents (TPC) were determined as galic acid (GA) equivalents by

standard curve equation y=0.0047x+0.0615 (r^2=0.998) and expressed in mg of GA/g of extract (Ismail *et al.,* 2004). TPC were found 21.84±0.61 only in DCM extract GA/g of extract (table 1).

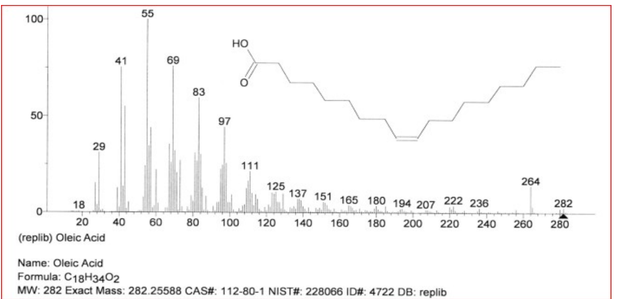

Fig. 10: Mass spectra and structure of Oleic acid.

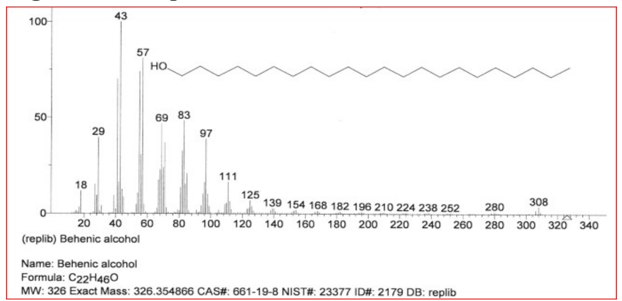

Fig. 11: Mass spectra and structure of Behenic alcohol.

The anti-oxidant activity of both dichloromethane and methanol extracts was very insignificant and having inhibition 5.52±2.66% and 19.20±1.21% (table 4).

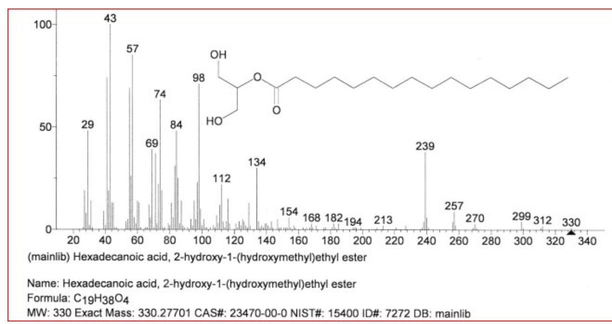

Fig. 12: Mass spectra and structure of Hexadecanoic acid, 2-hydroxy-1-(hydroxyl methyl) ethyl ester.

Through GC-MS analysis (David *et al.,* 2011; Hites *et al.,* 2016), the eight (08) compounds (1) 2,4-nonanedione (fig. 6), (2) Thymol (fig. 7), (3) n-hexadecanoic acid (fig. 8), (4) trans-13-Octadecenoic acid (fig. 9), (5) Oleic acid (fig. 10), (6) Behenic alcohol (fig. 11), (7) hexadecanoic acid, 2-hydroxy-1-(hydroxyl methyl) ethyl ester (fig. 12) and (8) 3‘,8,8’-trimethoxy-3-piperidyl-2,2’-binaphthalene-1,1’,4,4’-tetrone (fig. 13) were identified in dichloromethane extract (FHRD), whereas only one compound (1) cyclo propane botanic acid, 2-[[2-[[2-[(2-pentyl cyclo propyl) methyl] cyclo propyl]methyl]cyclo propyl]methyl]-, methyl ester (fig. 14) were identified in methanol extract (FHRM) of root part of *F. hamiltonii* (table 2).

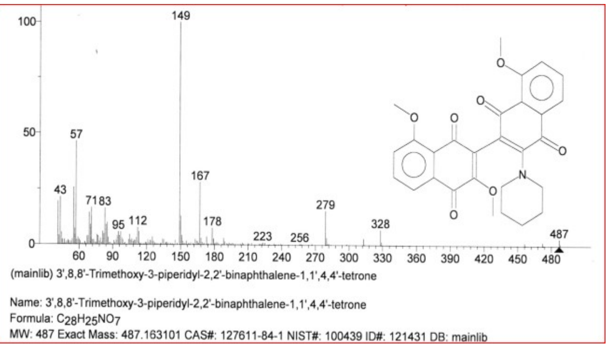

Fig.13: Mass spectra and structure of 3‘,8,8’-trimethoxy-3-piperidyl-2,2’-binaphthalene-1,1’,4,4’-tetrone.

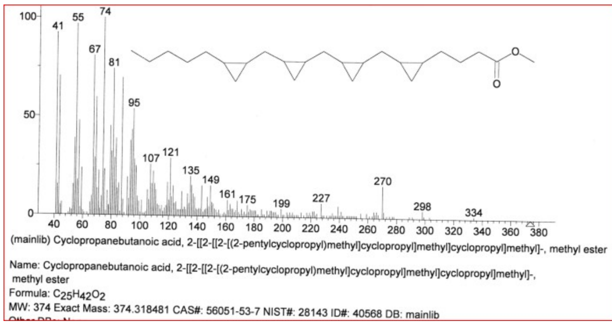

Fig. 14: Mass spectra and structure of Cyclo propane botanic acid, 2-[[2-[[2-[(2-pentyl cyclo propyl)methyl] cyclo propyl]methyl]cyclo propyl]methyl], methyl ester.

The dichloromethane extract of root parts of *F. hamiltonii* (FHRD) exhibited anti-cholinesterase inhibition value same for both anti-acetylcholinesterase and anti-butyrylcholinesterase which is 68.77±0.62% and IC50 405.41±0.21µmoles, anti-lipoxygenase (75.00±1.46% and IC50 291.70±2.45µmoles) and anti-chymotrypsin (69.64±0.01% and IC50 333.12±0.01µmoles) which are active and comparable to standards Eserine, Baicalein and Chymostatin respectively at moderate level, whereas anti-alpha glucosidase (16.45±1.13%) activity was very insignificant and found inactive.

The methanol extract of root parts of *F. hamiltonii* (FHRM) exhibited anti-cholinesterase (anti-acetylcholinesterase, anti-butyrylcholinesterase) with same inhibition 72.77±0.71% and IC_{50} 399.12±0.19 µmoles, anti-lipoxygenase (74.00±0.36% and IC50 323.50±2.19µmoles), anti-chymotrypsin (76.93±0.01% and IC50 238.57±0.01µmoles) and anti-alpha glucosidase (88.62±2.25% and IC50 152.95±1.84µmoles) activities.

The antibacterial activity of dichloromethane extract was insignificant against all five strains (*Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli*) with very less inhibition values 39.33±0.89%, 34.75±1.42%, 37.41±1.52%, 30.57±2.10%, 40.95±1.86%, respectively.

Table 2: Phytochemical constituent identified by GC-MS in dichloromethane extract of root part of *F. hamiltonii*.

S. No.	Compound Name	Mol. formula	Mol. Weight	Structure
1	2,4-Nonanedione	$C_9H_{16}O_2$	156	
2	Thymol	$C_{10}H_{14}O$	150	OH
3	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	OH
4	Trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	OH
5	Oleic acid	$C_{18}H_{34}O_2$	282	HO
6	Behenic alcohol	$C_{22}H_{46}O$	326	
7	Hexadecanoic acid, 2-hydroxy-1-(hydroxyl methyl) ethyl ester	$C_{19}H_{38}O_4$	330	OH HO
8	3‘,8,8’-trimethoxy-3-piperidyl-2,2’-binaphthalene-1,1’,4,4’-tetrone	$C_{28}H_{25}NO_7$	487	N

Table 3: Phytochemical constituent identified by GC-MS in methanol extract of root part of *F. hamiltonii*.

S.No.	Compound Name	Mol. formula	Mol. weight	Structure
1	Cyclo propane botanic acid, 2-[[2-[[2-[(2-pentyl cyclo propyl)methyl]cyclo propyl]methyl]cyclo propyl]methyl]-, methyl ester	$C_{25}H_{42}O_2$	374	

Table 4: Enzyme inhibition assays of dichloromethane and methanol extracts of root of *F. hamiltonii*.

S. No.	Enzyme inhibition assay	Dichloromethane Extract		Methanol Extract		Standard	
		Inhibition (%)	IC_{50} (µmoles)	Inhibition (%)	IC_{50} (µmoles)	Inhibition (%)	IC_{50} (µmoles)
1	Alpha glucosidase inhibition assay	16.45±1.13	Inactive	88.62± 2.25	152.95± 1.84	92.23± 0.14 Acarbose	38.25±0.12 Acarbose
2	Lipoxygenase inhibition assay	75.00±1.46	291.7±2.45	74.00±0.36	323.50±2.19	93.79±1.27 Baicalein	22.47±0.04 Baicalein
3	Chymotrypsin inhibition assay	69.64±0.01	333.12±0.01	76.93±0.01	238.57±0.01	93.50±0.91 Chymostatin	8.24± 0.11 Chymostatin
4	Antiacetylcholinesterase assay	68.77±0.62	405.41±0.21	68.77±0.62	405.41±0.21	91.29±1.17 Eserine	0.04±0.0001 Eserine
5	Antibutyrylchol-inesterase assay	72.77±0.71	399.12±0.19	72.77±0.71	399.12±0.19	91.29±1.17 Eserine	0.04±0.0001 Eserine
6	Antioxidant assay	5.52±2.66	Inactive	19.20±1.21	Inactive	83.68±3.76 Quercetin	16.96±0.14 Quercetin

Table 5: Antibacterial activity with MIC_{50} values of crude extracts of root part of *F. hamiltonii*.

Extract Code	Type	*Bacillus subtilis* (G+ve)	*Staphylococcus aureus* (G+ve)	*Pseudomonas aeruginosa,* (G-ve)	*Salmonella typhi* (G-ve)	*Escherichia coli* (G-ve)
FHRD	Inhibition* (%)	39.33±0.89	34.75±1.42	37.41±1.52	30.57±2.10	40.95±1.86
	MIC_{50} (µg/mL)	Inactive	Inactive	Inactive	Inactive	Inactive
FHRM	Inhibition (%)	64.44±2.89	70.98±1.57	62.92±2.74	57.81±5.00	62.41±2.14
	MIC_{50} (µg/mL)	12.83±0.38	11.01±0.43	12.61±0.63	11.69±0.33	12.83±0.10
Ciprofloxacin	Inhibition (%)	91.23±1.07	91.23±1.07	90.88±0.16	92.65±1.10	91.45±2.19
	MIC_{50} (µg/mL)	7.52±0.67	7.03±0.53	7.58±0.19	7.23±0.71	8.21±0.11

* Concentration is 20 µg/mL.

The antibacterial activity of methanol extract was significant against all five strains (*Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli*) at 64.44±2.89%, 70.98±1.57%, 62.92±2.74%, 57.81±5.00%, 62.41±2.14% and MIC50 12.83±0.38µg/mL, 11.01±0.43µg/mL, 12.61±0.63µg/mL, 11.69±0.33µg/mL, 12.83±0.10µg/mL and comparable to standard. Ciprofloxacin having inhibition 91.23±1.07%, 91.23±1.07%, 90.88±0.16%, 92.95±1.10%, 91.45±2.19% and MIC50 7.52±0.67µg/mL, 7.03±0.53µg/mL, 7.58±0.19µg/mL, 7.23±0.71µg/mL, 8.21±0.11µg/mL, respectively (table 5).

The alpha glucosidase inhibition assay of methanol extract was 88.62±2.25% that was about four-fold less potent than standard drug Acarbose. Lipoxygenase inhibition results for dichloromethane and methanol extracts were 75.0±1.46% (IC_{50}=291.7±2.45µg/mL) and 74.0±0.36% (IC_{50}=323.5±2.19µg/mL) that indicated that extracts of roots of *F. hamiltonii* were ten folds inhibitory at same concentration and comparable to standard Baicalein. Both the extracts inhibits at good level to enzymes Acetycholinesterase and Butyrylcholinesterase (68.77±0.62% and IC_{50}=405.41±0.21µg/mL), whereas methanol extract exhibited inhibition (72.77±0.71% with IC_{50}=399.12±0.19µg/mL).

The antibacterial activity along with MIC_{50} values of both dichloromethane and methanol extracts were evaluated against two gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus*) and three gram-negative (*Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli*). The only methanol extract showed significant bacterial growth inhibition (given in percentage in table 4) at concentration 20 µg/mL and MIC_{50} values comparable to standard ciprofloxacin.

DISCUSSION

The roots of *F. hamiltonii* were evaluated for secondary metabolites (alkaloids, glycosides, anthraquinones, flavonoids, saponins, steroids, leucoanthocyanins, coumarins and diterpenes) by phytochemical tests. The presence of glycosides, bounded anthraquinones, flavonoids, saponins, steroids, coumarins and diterpenes was confirmed. Occurrence of these phytochemical compounds indicates the therapeutic potential of the plant and provide a guideline to design a protocol for purification. Total flavonoid and phenolic contents were determined. Presence of saponins in the plant shows capability for diversified biological activities (Atta *et al*., 2001) and presence of steroidal compound confirm the antibacterial properties of the plant (Liu *et al.,* 2002).

Through GC-MS analysis, the eight (08) compounds (fig. 6 to 13) were identified in dichloromethane extract (FHRD), whereas only one (01) compound (fig. 14) identified in methanol extract (FHRM) of root part of *F. hamiltonii* (table 2).

The root part of *F. hamiltonii* was evaluated for anti-oxidant, anti-cholinesterase (anti-acetylcholinesterase, anti-butyrylcholinesterase), anti-lipoxygenase, anti-chymotrypsin, anti-alpha glucosidase and anti-bacterial activities to validate the floric use of root parts of the plant.

The products of lipoxygenases play a vital role in many medical disorders such as inflammation, bronchial asthma (Steinhilber *et al.,* 1999) and tumor angiogenesis (Nie *et al.,* 2002). Urease is of medical importance because it is involved regarding establishing the treatment of disease state like peptic ulcer, kidney stones, pyelonephritis and other diseases (Fishbein *et al.,* 1973). Therefore, the role of urease in the production of certain diseases is of much importance in pharmaceutical research (Robinson *et al.,* 1939). Urease enzyme is found in most of the organisms. It breaks down the urea to ammonium carbonate and is mostly used for determination of urea (Tarun *et al.,* 2009). Urea catalysis is carried out by urease forming carbonate anions and ammonium cations. Research is going on urea in many laboratories as it has gained much worth in biotechnology and medicine (Mobely, 1995).

Thus, the use of *F. hamlitonii* in herbal preparation can be helpful in starch digestion. The results of these enzymes (see table 4) give valuable importance as raw source of enzymes and metabolic importance of these enzymes in the plant reactions regarding production of different secondary metabolites.

The dichloromethane extract was good inhibitor of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes having IC_{50} values 405.41±0.21µg/mL (same for both). The methanol extract also exhibited inhibition with IC_{50} value 399.12±0.19 µg/mL. AChE and BChE are the enzymes involved in Alzheimer's disease. BChE is valuable when an individual is exposed to the poisonous compounds directly acting on the acetylcholine binding site, which results in loss of functions AChE which further leads to muscle paralysis, seizure and can cause death by asphyxiation. BChE detoxifies them before reaching the physiologically important target sites of AChE. Hence, BChE is considered as an endogenous scavenger of anticholinesterase compounds. To roll out prevention from diseases by the Inhibition of BChE enzyme, for this intention extracts of roots of *F. hamiltonii* were tested for their inhibition so that they can be used later to cure Alzheimer and dementia related diseases (Dos *et al.,* 2006).

Lipoxygenase (LOXs) inhibition assay was significant in the way that both dichloromethane and methanol extracts exhibited inhibitions of LOXs with IC_{50} values as 291.7±2.45µg/mL and 323.5±2.19µg/mL, respectively. LOXs are extensively present in plants, fungi and the animals, are members of large monomeric protein family with non-heme iron co-factor containing dioxygenase group (Gardner *et al.,* 1991). In plants, the hydroperoxide fatty acids, (mainly derived from linolenic and linoleic acids) are further metabolized into physiologically active lipid-breakdown products, such as growth regulators and signal transduction molecules like, traumatinabscissic acid and jasmonic acid (Vick *et al.,* 1993). In different plant species, LOXs are present as multiple isoforms or isozymes, signifying that each one may play diverse functions within the plant. The products of the reaction are conjugated cis, trans hydroperoxy derivatives, which are highly reactive and toxic to cells. They are rapidly metabolized to non-toxic but physiologically- active components. It has been found that these LOX products play a vital role in variety of disorders such as bronchial asthma, inflammation and tumor angiogenesis. The results of LOXs inhibition prove the presence of important phytochemical present in the crude extract and is related to its traditional uses such as treating asthma and inflammations (Fischer *et al.,* 1999).

To justify the traditional diabetic use of *F. hamiltonii*, *in vitro* alpha glucosidase inhibition assay was done. The IC_{50} value 152.95±1.84µg/mL of methanol extract of root part is significant which is comparable with IC_{50} value (38.25±0.12µg/mL) of standard acarbose (table 4). The alpha glucosidase is enzyme have role in the hydrolysis of disaccharides into glucose, thus its inhibition can suppress the postprandial hyperglycemia and leads to useful invention for management of diabetes type II (Choudhary *et al.,* 2011; Kim *et al.,* 2000).

The percentage inhibition of bacterial growth along with minimum inhibitory concentrations of both

dichloromethane and methanol extracts of roots of *F. hamiltonii* evaluated against two gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus*) and three gram-negative (*Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli*). Natural products-based drugs are gaining popularity because of their no side effects and low cost. Pathogenic bacteria are developing resistance against antibiotics (Kaspady *et al.,* 2009) and imposing threat to human kind. The MIC_{50} Value of methanol extract was 11.01±0.43 against *Staphylococcus aureus* which is close to MIC_{50} value of standard Ciprofloxacin that is 7.03±0.51 which proves the traditional use of this plant having potential to treat food poisoning, urinary tract and skin infections (boils, Impetigo, Cellulitis) caused by this pathogen. Furthermore, MIC_{50} values (Valsaraj *et al.,* 1997) of methanol extract of root part of *F. hamiltonii* against five pathogenic bacteria are significant and comparable to standard ciprofloxacin (table 4).

The DPPH scavenging ability (RSA) of plant extracts of root part of *F. hamiltonii*, the results of our study regarding % RSA of both dichloromethane and methanol extracts were not significant (table 4).

CONCLUSION

This work is first scientifically proven report on root part of *F. hamiltonii* that justifies the traditional uses of plant for diabetes and infectious diseases. For the first time, nine (09) compounds (fig. 6 to 14) were reported in the root part of *F. hamiltonii*. It was observed during this study that dichloromethane extract of root parts of *F. hamiltonii* exhibited anticholinesterase, anti-lipoxygenase and anti-chymotrypsin activities. And, methanol extract exhibited acetylcholinesterase, anti-lipoxygenase, anti-chymotrypsin, anti-alpha glucosidase, anti- and antibacterial activities. It is suggested by this work that the *F. hamiltonii* can be effectively used against diabetes, inflammation and skin diseases causing pathogenic bacteria.

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