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PHYTOCHEMICAL SCREENING AND IN VITRO EVALUATION OF THE ANTI-INFLAMMATORY AND ANTIDIABETIC PROPERTIES OF *SINOMENIUM ACUTUM* RHIZOME EXTRACT

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Abstract:
Diabetes and inflammation are leading causes of fatality worldwide, accounting for millions of deaths every year. Although many drugs have been developed to treat these conditions effectively, some pharmacological incompatibilities could happen simultaneously. Therefore traditional natural product drugs to fight these conditions are more beneficial and are still urgently needed. Hence we have biologically evaluated Chinese traditional herb Sinomenium acutum rhizome extracts as potential antidiabetic and anti-inflammatory natural product by using suitable in vitro bioassays. The tested extracts of Sinomenium acutum rhizome possess in-vitro anti-inflammatory and antidiabetic activities.

Keywords: *Diabetes, Inflammation, Sinomenium acutum, Chinese herbal medicine*

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INTRODUCTION:

Natural plant products are one of the sources that have been used throughout by the human for various indications since a long ago. The history of use of herbs can be traced back to the emperor Shen Nong who identified 100 of herbs and imparted the knowledge on usage of herbs in diet and therapy to Chinese people, hence herbal medicine became an integral part of Chinese culture and medical practice until now. The traditional Chinese *materia medica*, also known as *Bencao* Gangmu, a complete and comprehensive medical book of traditional Chinese medicine, includes all the minerals and animal parts as well as herbs that were believed to have medicinal properties.

A novel application of plants in the process of treatment was established in the human history and formed as the basis of much of modern medicine. About 25% of drugs prescribed worldwide are derived from plants. In fact, many conventional drugs are found from plant sources, such as aspirin from willow bark, digoxin from foxglove, quinine from cinchona bark, and morphine from the opium poppy. Dominantly as one of the ancient herbal traditions, Chinese herbalism is currently practiced until now. Based on the concepts of yin and yang and of *Qi* energy, Chinese herbs describe the patient in term of qualities such as "cooling" (yin) or "stimulating" (yang) and are used, often in combination, whether are deficiencies or excesses in the patient. However, modern Western herbalism focuses on the effects of herbs on individual body systems. For instance anti-inflammatory, hemostatic, expectorant, antispasmodic, or other properties are treated by the use of herb . (Vickers A. *et. al.*, 2001)Thus, it is a good suggestion to explore the use of natural products as a part of treatment in various kinds of diseases.

RESEARCH METHODOLOGY:

Materials

Collection of plant material

The rhizomes of *Sinomenium acutum* was procured from the local herbal shop in October 2015.

Plant authentication

The rhizomes of *Sinomenium acutum* were authenticated (Ref: UPM/IBS/UB/H88-15) by Dr. Shamsul Khamis, Botanist and Senior Science Officer, Biodiversity Unit, UPM (University Putra Malaysia).

Reagents and chemicals

1 Kg of shade dried rhizomes powder of *Sinomenium acutum (Thumb.)*, polar and non-polar solvents such as hexane, chloroform, dichloromethane, acetone, acetonitrile, methanol, ethanol, diethyl ether were procured from AMU lab department, other chemicals such as Hexane (1 ltr) , chloroform(1 ltr), ethyl acetate (1 ltr), 80%methanol (2 ltr), Iodine solution, Ninhydrin's solution, Hydrochloric acid (HCl) solution, Mayer's reagent, Wagner's reagent, Iron (III) chloride solution, Distilled water, Magnesium ribbon, Sodium nitropruside solution (dilute and concentrated), Sodium hydroxide solution, Sulphuric acid solution, Chloroform solution, Silica Gel-G, Toluene solution, Acetone solution, Sodium Bicarbonate solution, Metformin HCl, Naproxen and other generally used laboratory glassware and equipment have also been obtained from regular chemical indent supply at AMU lab department.

Equipments required

Beakers, filter funnel, micropipette, Whattman No.3 filter paper, UV-chamber, rotary evaporator, UV visible spectrophotometer, test tube, pipette, pre coated TLC plates, vernier calliper, mortar and pastel, glass rod, incubator, cuvattes, separating funnel, capillary tubes, porcelain dishes, gloves, mask, measuring cylinder, centrifuge, vortex mixer, filter paper, muslin cloth.

Methods

Extraction method: Cold maceration

Cold maceration is a liquid-solid type solvent extraction method was used for extraction. In this process, the whole or coarsely powdered crude drug was placed in a stoppered container with 80% methanol and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has been dissolved. The mixture was then strained, the marc (the damp solid material) was pressed, and the combined liquids were clarified by filtration or decantation after standing for obtaining crude extract. The marc was re-extracted with another solvent basing on order of polarity hexane, chloroform, ethyl acetate (Barkat Ali Khan et al; 2012., Xun Yan et al 2008, Harbone J.B 1998). The dark residue obtained from different solvent extractions was subjected for evaluation of selected bioassays.

Biological evaluation

In vitro anti-inflammatory bioassay (Inhibition of bovine serum albumin (BSA) denaturation assay)

Anti-denaturation assay was conducted as described by Biswakanth et al. with slight modifications. Different extracts of Sinomenium acutum rhizomes were dissolved in minimum amount of dimethylsulfoxide (DMSO) and diluted with phosphate buffer saline (PBS, pH 7.4 ± 0.02). Final concentration of DMSO in all solution was less than or equal to 1%. Test solution (1mL) containing different concentrations of extracts of *Sinomenium acutum* were taken in test tubes and mixed with 1 mL of 1mM bovine serum albumin solution in phosphate buffer. The final concentration of each extract in 2 mL of reaction mixture was 5, 10, 25, 50, 100 µg/mL respectively.

Distilled water (2 mL) served as control. Test tubes were incubated at 30 ºC in an incubator for 15 min and then heated at 65 °C for 10 min. Test tubes were cooled and the absorbance was measured at 660 nm using UV-Vis Spectrophotometer (Jasco V-630). Naproxen was used as the standard drug and treated similarly to determine the absorbance. The experiment was performed in triplicate. Percentage inhibition of bovine serum albumin protein denaturation was calculated by using the following formula:

% inhibition = 100 × [Vt / Vc – 1]

Where, Vt = absorbance of test sample, Vc = absorbance of control

In vitro antidiabetic bioassay (Glucose uptake by yeast cells bioassay)

Yeast cells were prepared according to the method of Cirillo (Cirillo, V. P. et al., 1962). Commercial baker's yeast was washed by repeated centrifugation (4200 r/min, 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of the *Sinomenium acutum* rhizomes extracts were added to 1 mL of glucose solution (1-5 mg/mL) and incubated together for 10 min at 37 °C. The reaction was started by adding 100 µL of yeast suspension, vortexed and further incubated at 37 °C for 60 min. After 60 min, the tubes were centrifuged (3 800 r/min, 5 min) and glucose was estimated in the supernatant. The percent increase in glucose uptake by yeast cells was calculated using UV method based on the absorbance of blank reaction (containing all reagents except the test sample) and test sample.

UV method for estimation of glucose

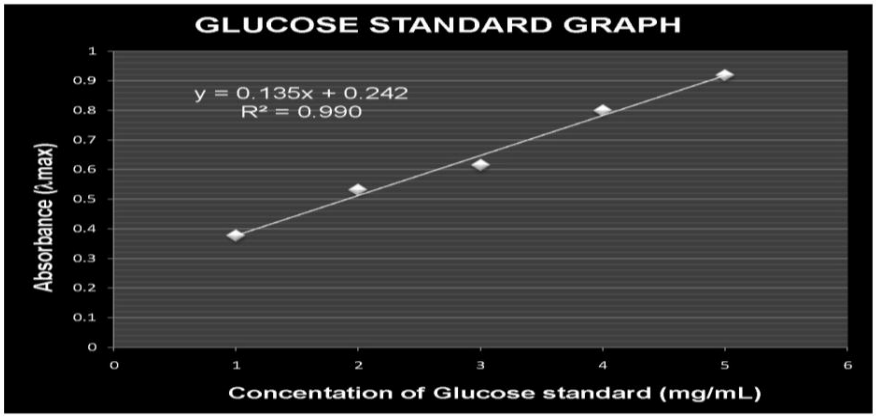
Glucose estimation was done according to the by modification of sulfuric acid-UV as described by Berhe et al., 2013. A 1 mL of aliquot of glucose concentrations 1, 2, 3, 4, 5 mg/mL respectively was rapidly mixed with 1 mL of concentrated sulfuric acid in a test tube and vortexed for 10 sec. The temperature of the reaction mixture was raised rapidly within 10 sec after addition of sulfuric acid. Then the solution was added with 2 mL of water and cooled in ice for 2 min and brought it back to the room temperature. Finally, UV light absorption at 315 nm was recorded using UV-double beam spectrophotometer (Jasco V-630). The glucose content was estimated from a standard curve prepared with standard solutions. Standard glucose solutions (1-5 mg/mL) were prepared using dilution method. The calibration curve for glucose estimations was plotted with a correlation coefficient (r2) of 0.99, indicating acceptable precision and accuracy of the method for the estimation of glucose.

Table 1: Standard plots for estimation of glucose using sulfuric acid based UV method at 315 nm.

S.No	Concentration of glucose standard solution	Absorbance response (λmax) at 315 nm*
1	Blank	0.2653 ± 0.11
2	Glucose standard 1 (mg/mL)	0.3773 ± 0.14
3	Glucose standard 2 (mg/mL)	0.5306 ± 0.43
4	Glucose standard 3 (mg/mL)	0.6139 ± 0.24
5	Glucose standard 4 (mg/mL)	0.7991 ± 0.51
6	Glucose standard 5 (mg/mL)	0.9185 ± 0.74

*Results were expressed as mean±SEM (n=3)

Fig 1: Standard graph for estimation of glucose using sulfuric acid based UV method at 315 nm.



Concentration (mg/mL) = (Absorbance) - 0.242
0.135

Phytochemical screening

Small amount of each extract was investigated to find the presence of different phytochemicals such as carbohydrates, proteins, alkaloids, phenols, tannins, saponins, flavonoids, glycosides, steroids and terpenoids respectively by using following standard methods.

Preliminary identification:

Phytochemical analysis will be done following standard methods (Tyler 2013 and Rohit kumar Bargah 2015)

Carbohydrates -Iodine test

Crude extract will be mixed with 2mL of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrate.

Proteins- Millon's test

Crude extract when mixed with 2mL of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein. Ninhydrin test Crude extract when boiled with 2mL of 0.2% solution of Ninhydrin, violet colour appeared suggesting the presence of amino acid and proteins.

Alkaloids

Crude extract will be mixed with 2mL of 1% HCl and heated gently. Mayer's And Wagner's reagents will be then added to the mixture. Turbidity of the resulting precipitate will be taken as evidence for the presence of alkaloids.

Phenols and tannins

Crude extract will be mixed with 2mL of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins.

Saponins

Crude extract will be mixed with 5mL of distilled water in a test tube and it will be shaken vigorously. The formation of stable foam will be taken as an indication for the presence of saponins.

Flavonnoids- Shinoda test

Crude extract will be mixed with few fragments of magnesium ribbon and concentrated HCl will be added drop wise. Pink scarlet colour appeared after few minutes which indicated the presence of flavonoids.

Glycosides

2 mL of crude extracts will be added with dilute hydrochloride acid and followed by 2 ml Sodium nitropruside in pyridine and sodium hydroxide solution. Formation of pink to blood red colour indicates the presence of Cardiac glycosides.

Steroids- Salkowski's test

2mL of crude extract will be dissolved in 2mL of chloroform and 2mL concentrated sulphuric acid will be added in it. A red color produced in the lower chloroform layer indicates the presence of steroids.

Terpenoids

Crude extract will be dissolved in 2mL of chloroform and evaporated to dryness. To this, 2mL of concentrated H_2SO_4 will be added and heated for about 2 minutes. A greyish colour indicated the presence of terpenoids.

Thin layer chromatography (TLC) profile of bioactive extracts:

Using a micropipette, about 10µmL of bioactive extracts were loaded gradually over the pre-coated TLC-G_{254} plate and air dried. Then the plates were eluted using different solvent systems ranging from non-polar to polar solvent system in different degrees of polarity. The chromatograms were observed under UV-chamber and were photographed. The R_f values were calculated for all the compounds observed under UV light by using the following formula. The R_f values were falling within the range of 0.01 to 0.99.

$$Rf = \frac{Distance\ travelled\ by\ the\ solute\ (cm)}{Distance\ travelled\ by\ the\ solvent\ (cm)}$$

RESULTS:

Biological Evaluation

Anti-Inflammatory Bioassay (In vitro bovine serum albumin denaturation)

Table 2: Influence of *Sinomenium acutum* rhizomes extracts against in vitro bovine serum albumin denaturation (anti-inflammatory bioassay)

S.No	Extract / Standard	Concentration	% Inhibition*
1.	**Hexane Extract (HE)**	5 µg/mL	NS
		10 µg/mL	NS
		25 µg/mL	NS
		50 µg/mL	NS
		100 µg/mL	13.01 ± 0.44
2.	**Chloroform Extract (CE)**	5 µg/mL	NS
		10 µg/mL	NS
		25 µg/mL	NS
		50 µg/mL	11.34 ± 0.71
		100 µg/mL	28.54 ± 0.44

Continue…….

3.	**Ethylacetate Extract (EE)**	5 µg/mL	NS
		10 µg/mL	18.17 ± 0.31
		25 µg/mL	31.45 ± 0.01
		50 µg/mL	49.14 ± 0.17
		100 µg/mL	60.44 ± 0.41
4.	**Acetone Extract (AE)**	5 µg/mL	18.31 ± 1.10
		10 µg/mL	32.64 ± 0.17
		25 µg/mL	58.14 ± 0.12
		50 µg/mL	71.51 ± 0.13
		100 µg/mL	85.04 ± 0.11
5.	**Methanol Extract (ME)**	5 µg/mL	NS
		10 µg/mL	17.44 ± 0.21
		25 µg/mL	26.31 ± 0.41
		50 µg/mL	54.54 ± 0.14
		100 µg/mL	79.21 ± 0.22
6.	**Naproxen**	5 µg/mL	21.01 ± 0.74
		10 µg/mL	32.41 ± 0.24
		25 µg/mL	55.32 ± 0.81
		50 µg/mL	67.52 ± 0.01
		100 µg/mL	89.11 ± 0.14

*NS: Not significant (<10%), * Results were expressed as mean±SEM (n=3)*

Antidiabetic Bioassay (In vitro glucose uptake by yeast cells)

Table 3: Influence of *Sinomenium acutum* rhizomes extracts against in vitro glucose uptake by yeast cells (antidiabetic bioassay)

S.No	Extract / Standard	Concentration	% Enhancement*
1.	**Hexane Extract (HE)**	5 µg/mL	NS
		10 µg/mL	NS
		25 µg/mL	NS
		50 µg/mL	NS
		100 µg/mL	NS
2.	**Chloroform Extract (CE)**	5 µg/mL	NS
		10 µg/mL	NS
		25 µg/mL	NS
		50 µg/mL	NS
		100 µg/mL	NS
3.	**Ethylacetate Extract (EE)**	5 µg/mL	NS
		10 µg/mL	NS
		25 µg/mL	16.12 ± 0.33
		50 µg/mL	21.32 ± 0.02
		100 µg/mL	25.07 ± 0.12
4.	**Acetone Extract (AE)**	5 µg/mL	NS
		10 µg/mL	15.11 ± 0.82
		25 µg/mL	28.17 ± 0.33
		50 µg/mL	47.81 ± 0.04
		100 µg/mL	63.19 ± 0.48
5.	**Methanol Extract (ME)**	5 µg/mL	NS
		10 µg/mL	NS
		25 µg/mL	11.31 ± 0.41
		50 µg/mL	27.54 ± 0.14
		100 µg/mL	48.21 ± 0.22
6.	**Metformin HCl**	5 µg/mL	13.44 ± 0.74
		10 µg/mL	21.07 ± 0.41
		25 µg/mL	38.64 ± 0.14
		50 µg/mL	55.71 ± 0.48
		100 µg/mL	74.86 ± 0.13

*NS: Not significant (<10%), * Results were expressed as mean±SEM (n=3)*

Phytochemical Screening

Table 4: Phytochemical screening of *Sinomenium acutum* rhizomes extracts

S.No	Phytochemical	Hexane Extract (HE)	Chloroform Extract (CE)	Ethyl acetate Extract (EE)	Acetone Extract (AE)	Methanol Extract (ME)
1.	Alkaloids	ND	ND	+	+	+
2.	Carbohydrates	ND	ND	+	+	+
3.	Flavonoids	ND	ND	-	-	-
4.	Glycosides	ND	ND	+	+	+
5.	Phenols	ND	ND	+	+	+
6.	Proteins	ND	ND	-	-	-
7.	Saponins	ND	ND	-	-	-
8.	Steroids	ND	ND	-	-	-
9.	Tannins	ND	ND	-	-	-
10.	Terpenoids	ND	ND	-	-	-

*Not determined

Table 5: Retention factor (R_f) values of compounds separated from the bioactive EE extract in different mobile phase solvent systems

Mobile Phase	No. of Eluants	Distance travelled by the sample (cm)			Distance travelled by the solvent (cm)	R_f Value		
		Ethylacetate Extract				Ethylacetate Extract		
100% Hexane	1	0	0	0	14.7	0	0	0
5% EtOAc /Hex	1	11.5			14.7	0.78		
	2	10.5	9.8	9.1	14.7	0.71	0.67	0.62
	3	8.9			14.7	0.61		
	4	4.5			14.7	0.31		
	5	2.5			14.7	0.17		
	6	1.4			14.7	0.10		
	7	0.7			14.7	0.05		
	8	0.4			14.7	0.03		
	9	0	0	0.7	14.7	0	0	0.05
10% EtOAc /Hex	1	6.4	6.6	6.9	14.9	0.43		
	2	6.1			14.9	0.41		
	3	4.4			14.9	0.30		
	4	3.7	3.6	3.6	14.9	0.25	0.24	0.24
	5	2.9			14.9	0.19		
	6	0			14.9	0		
20% EtOAc /Hex	1	9.8			14.8	0.66		
	2	9.4	9.4	9.4	14.8	0.64	0.64	0.64
	3	8			14.8	0.54		
	4	5.3			14.8	0.36		
	5	3.7			14.8	0.25		
	6	3.4			14.8	0.23		
	7	3	2.8	2.8	14.8	0.20	0.19	0.19
	8	0			14.8	0		

Continue……

30% EtOAc /Hex	1	13.8			14.7	0.94		
	2	13.4	13.9	14	14.7	0.91	0.95	0.95
	3	12.9			14.7	0.88		
	4	11.7			14.7	0.80		
	5	10.3			14.7	0.70		
	6	9.4	8.8	8.6	14.7	0.64	0.60	0.59
	7	6.6			14.7	0.45		
	8	0			14.7	0.00		
50% EtOAc /Hex	1	13.9	13.9	14	15.4	0.90	0.902597	0.909091
	2	12.9			15.4	0.84		
	3	12.4			15.4	0.81		
	4	11.2			15.4	0.73		
	5	9	9	9	15.4	0.58	0.58	0.58
	6	8.6			15.4	0.56		
	7	7.8			15.4	0.51		
	8	4.6			15.4	0.30		
70% EtOAc /Hex	1	13.8	13.9	13.8	14.9	0.93	0.93	0.93
	2	12.9			14.9	0.87		
	3	12.1			14.9	0.81		
	4	10.9			14.9	0.73		
	5	9.7	9.5	9.4	14.9	0.65	14.59	0.63
	6	8.7			14.9	0.58		
	7	0			14.9	0		
100% EtOAc	1	11.8	12.6	12.8	14.8	0.80	0.85	0.86
	2	11.1			14.8	0.75		
	3	10.7			14.8	0.72		
	4	10.4			14.8	0.70		
	5	9.8			14.8	0.66		
	6	7.3	7.4	7.3	14.8	0.49	0.50	0.49
	7	5.4			14.8	0.36		
	8	4.2			14.8	0.28		
	9	1.3			14.8	0.09		
	10	0			14.8	0		
1% MeOH /EtOAc	1	12.3	12.2	12.3	14.8	0.83	0.82	0.83
	2		10.4		14.8		0.70	
	3		10		14.8		0.68	
	4		9.3		14.8		0.63	
	5	7.1	6.9	7	14.8	0.48	0.47	0.47
	6		5		14.8		0.34	
	7		3.8		14.8		0.26	
5% MeOH /EtOAc	1	12	12.2	12.4	14.7	0.82	0.83	0.84
	2	10.7			14.7	0.73		
	3	10.3			14.7	0.70		
	4	9.5			14.7	0.65		
	5	8.5			14.7	0.58		
	6	7	6.9	7	14.7	0.48	0.47	0.48
	7	4.8			14.7	0.33		

Continue……

10% MeOH /EtOAc	1	12.2	12.1	12.2	14.8	0.82	0.82	14.8
	2	11.2			14.8	0.76		
	3	10.7			14.8	0.72		
	4	10.3			14.8	0.70		
	5	9.7			14.8	0.66		
	6	7	6.8	6.9	14.8	0.47	0.46	0.47
	7	4.7			14.8	0.32		
	8	3.8			14.8	0.26		
	9	0			14.8	0		
30% MeOH /EtOAc	1	12.5	12.3	12.5	14.7	0.85	0.84	0.85
	2	11.4			14.7	0.78		
	3	10.5			14.7	0.71		
	4	10			14.7	0.68		
	5	9.1			14.7	0.62		
	6	7.5			14.7	0.51		
	7	5.7	5.7	5.7	14.7	0.39	0.39	0.39
	8	3.4			14.7	0.23		
50% MeOH /EtOAc	1	12.6	12.4	12.7	14.9	0.85	0.83	0.85
	2	11.8			14.9	0.79		
	3	10.8			14.9	0.72		
	4	10.1			14.9	0.68		
	5	9.2			14.9	0.62		
	6	8.5			14.9	0.57		
	7	7			14.9	0.47		
	8	5	4.8	4.8	14.9	0.34	0.32	0.32
	9	2.3			14.9	0.15		
	10	1.6			14.9	0.11		
	11	0			14.9	0		
75% MeOH /EtOAc	1	12.5	12.5	12.5	14.8	0.84	0.84	0.84
	2	11.5			14.8	0.78		
	3	10			14.8	0.67		
	4	9.3			14.8	0.63		
	5	8.5			14.8	0.57		
	6	7			14.8	0.47		
	7	3.9	3.8	3.8	14.8	0.26		
	8	1.3			14.8	0.09		
	9	0			14.8	0		
100% MeOH /EtOAc	1	12.5	12.5	12.5	15	0.83	0.83	0.83
	2	10.9			15	0.73		
	3	9.1			15	0.61		
	4	8.2			15	0.55		
	5	7.7			15	0.51		
	6	7.3			15	0.49		

Continue……

	7	6.5			15	0.43		
	8	6			15	0.40		
	9	5.6			15	0.37		
	10	3.9			15	0.26		
	11	2.7	2.7	2.7	15	0.18	0.18	0.18
	12	0.6			15	0.04		
	13	0			15	0		

Table 6: Retention factor (R_f) values of compounds separated from the bioactive AE extract in different mobile phase solvent systems

Mobile Phase	No. of Eluants	Distance travelled by the sample (cm)			Distance travelled by the solvent (cm)	R_f Value		
		Acetone Extract				Acetone Extract		
100% Hexane	1	0	0	0	14.7	0	0	0
5% EtOAc /Hex	1	11.3			14.7	0.77		
	2	10	10.5	10.8	14.7	0.68	0.71	0.73
	3	8.2			14.7	0.56		
	4	4			14.7	0.27		
	5	2.2			14.7	0.15		
	6	1.2			14.7	0.08		
	7	0.7			14.7	0.05		
	8	0.4			14.7	0.03		
	9	0	0.3	0.2	14.7	0	0.02	0.01
10% EtOAc/Hex	1	7.7	7.8	7.7	14.9	0.52	0.52	0.52
	2	7.5			14.9	0.50		
	3	7.4			14.9	0.50		
	4	5.6			14.9	0.38		
	5	4	3.9	4.1	14.9	0.27	0.26	0.28
	6	3.5			14.9	0.23		
	7	0	0	0	14.9	0	0	0
20% EtOAc/Hex	1	9.9			14.8	0.67		
	2	9.6	9.5	9.4	14.8	0.65	0.64	0.64
	3	8.4			14.8	0.57		
	4	5.5			14.8	0.37		
	5	3.4			14.8	0.23		
	6	2.9	3	3.1	14.8	0.20	0.20	0.21
	7	0			14.8	0		
30% EtOAc /Hex	1	13.4	13.3	13.7	14.7	0.91	0.90	0.93
	2	12.9			14.7	0.88		
	3	12.4			14.7	0.84		
	4	11.1			14.7	0.76		
	5	10			14.7	0.68		
	6	9	9.4	9.9	14.7	0.61	0.64	0.67
	7	8.5			14.7	0.58		
	8	7.8			14.7	0.53		
	9	7.5			14.7	0.51		

Continue……

	10	0			14.7	0.00		
50% EtOAc/Hex	1	13.8	13.6	13.5	15.4	0.90	0.88	0.88
	2	13			15.4	0.84		
	3	12.5			15.4	0.81		
	4	11.3			15.4	0.73		
	5	9	8.9	8.9	15.4	0.58	0.58	0.58
	6	8.6			15.4	0.56		
	7	7.9			15.4	0.51		
	8	4.9			15.4	0.32		
	9	0			15.4	0		
70% EtOAc/Hex	1	13.2	13.4	13.4	14.9	0.89	0.90	0.90
	2	12.6			14.9	0.85		
	3	12			14.9	0.81		
	4	11.7			14.9	0.79		
	5	10.6			14.9	0.71		
	6	9.3	9.4	9.6	14.9	0.62	0.63	0.64
	7	8.7			14.9	0.58		
	8	8.1			14.9	0.54		
	9	5			14.9	0.34		
	10	0			14.9	0		
100% EtOAc	1	12.3	12.2	12.1	14.8	0.83	0.82	0.82
	2	11.2			14.8	0.76		
	3	10.5			14.8	0.71		
	4	9.8			14.8	0.66		
	5	7.2	7.2	7.4	14.8	0.49	0.49	0.50
	6	5.2			14.8	0.35		
	7	4.1			14.8	0.28		
	8	1.5			14.8	0.10		
	9	0			14.8	0		
1% MeOH/EtOAc	1	11.9	11.9	12.4	14.8	0.80	0.80	0.84
	2	10.5			14.8	0.71		
	3	9.8			14.8	0.66		
	4	9.4			14.8	0.64		
	5	8.5			14.8	0.57		
	6	8.1			14.8	0.55		
	7	6.4	6.6	6.8	14.8	0.43	0.45	0.46
	8	4.7			14.8	0.32		
	9	3.6			14.8	0.24		
	10	1.3			14.8	0.09		
	11	0.5			14.8	0.03		
	12	0			14.8	0		
5%MeOH/EtOAc	1	11.7	11.9	11.6	14.7	0.80	0.81	0.79
	2	10.8			14.7	0.73		

Continue……

	3	10.2			14.7	0.69		
	4	9.6			14.7	0.65		
	5	8.4			14.7	0.57		
	6	7.7			14.7	0.52		
	7	6.9	6.9	7	14.7	0.47	0.47	0.48
	8	4.8			14.7	0.33		
	9	3.6			14.7	0.24		
	10	1.2			14.7	0.08		
	11	0			14.7	0		
10%MeOH/EtOAc	1	11.9	12.4	12	14.8	0.80	0.84	0.81
	2	10.8			14.8	0.73		
	3	10.6			14.8	0.72		
	4	10.2			14.8	0.69		
	5	9.5			14.8	0.64		
	6	8.1			14.8	0.55		
	7	6.7	6.8	7	14.8	0.45	0.46	0.47
	8	4.5			14.8	0.30		
	9	3.3			14.8	0.22		
	10	1.2			14.8	0.08		
	11	0			14.8	0		
30%MeOH/EtOAc	1	11.1	11.4	11.4	14.7	0.76	0.78	0.78
	2	10.5			14.7			
	3	9.9			14.7	0.67		
	4	9.2			14.7	0.63		
	5	8.6			14.7	0.59		
	6	6.5			14.7	0.44		
	7	5.4	5.4	5.4	14.7	0.37	0.37	0.37
	8	3.4			14.7	0.23		
	9	2.4			14.7	0.16		
	10	0.7			14.7	0.05		
50%MeOH/EtOAc	1	12.5	12.6	12.7	14.9	0.84	0.85	0.85
	2	11.5			14.9	0.77		
	3	10.5			14.9	0.70		
	4	9.7			14.9	0.65		
	5	8.8			14.9	0.59		
	6	8			14.9	0.54		
	7	6.5			14.9	0.44		
	8	4.6	4.7	4.5	14.9	0.31	0.32	0.30
	9	2.2			14.9	0.15		
	10	1.5			14.9	0.10		
	11	0			14.9	0		
75%MeOH/EtOAc	1	12.1	11.8	11.7	14.8	0.82	0.80	0.79

Continue……

	2	11			14.8	0.74		
	3	9.9			14.8	0.67		
	4	9			14.8	0.61		
	5	8.8			14.8	0.59		
	6	7.8			14.8	0.53		
	7	6.7			14.8	0.45		
	8	5.6			14.8	0.38		
	9	4.2			14.8	0.28		
	10	3.6	3.6	3.6	14.8	0.24	0.24	0.24
	11	1.2			14.8	0.08		
	12	0.9			14.8	0.06		
	13	0			14.8	0		
100% MeOH	1	11.6	11.8	11.7	15	0.77	0.79	0.78
	2	10.3			15	0.69		
	3	9			15	0.60		
	4	8.1			15	0.54		
	5	7.5			15	0.50		
	6	7.2			15	0.48		
	7	5.9			15	0.39		
	8	5.5			15	0.37		
	9	4.5			15	0.30		
	10	3.7			15	0.25		
	11	2.6	2.7	2.6	15	0.17	0.17	0.17
	12	0.6			15	0.04		
	13	0			15	0		

Table 7: Retention factor (R_f) values of compounds separated from the bioactive ME extract in different mobile phase solvent systems

Mobile Phase	**No. of Eluants**	**Distance travelled by the sample (cm)**			**Distance travelled by the solvent (cm)**	**R_f Value**		
		Methanol Extract				**Methanol Extract**		
100% Hexane	1	0	0	0	14.7	0	0	0
5% EtOAc/Hex	1	10.6			14.7	0.72		
	2	8	7.8	8.3	14.7	0.54	0.53	0.56
	3	6.4			14.7	0.44		
	4	2.3			14.7	0.16		
	5	1.4			14.7	0.10		
	6	1.1	1.1	0.6	14.7	0.07	0.07	0.04
	7	0.8			14.7	0.05		
	8	0			14.7	0		
10% EtOAc/Hex	1	7.3	7.5	7.6	14.9	0.49	0.50	0.51
	2	7			14.9			

Continue……

	3	6.7			14.9			
	4	3.8	3.8	3.8	14.9	0.26	0.26	0.26
	5	3.3			14.9			
	6	0			14.9			
20% EtOAc/Hex	1	9.8			14.8	0.66		
	2	9.5	9.5	9.5	14.8	0.64	0.64	0.64
	3	7.9			14.8	0.53		
	4	5			14.8	0.34		
	5	3.4			14.8	0.23		
	6	2.6	2.6	2.7	14.8	0.18	0.18	0.18
	7	0			14.8	0.00		
30%EtOAc/Hex	1	13.1	13.9	13.9	14.7	0.89	0.95	0.95
	2	12.6			14.7	0.86		
	3	12.1			14.7	0.82		
	4	10.7			14.7	0.73		
	5	8.8	8.8	8.5	14.7	0.60	0.60	0.58
	6	7.6			14.7	0.52		
	7	7.8			14.7	0.53		
	8	6.2			14.7	0.42		
	9	0			14.7	0.00		
50%EtOAc/ Hex	1	13.7	13.9	13.9	15.4	0.89	0.90	0.90
	2	12.9			15.4	0.84		
	3	11.3			15.4	0.73		
	4	9.2	9.2	9.2	15.4	0.60	0.60	0.60
	5	8.7			15.4	0.56		
	6	7.9			15.4	0.51		
	7	4.6			15.4	0.30		
70%EtOAc/Hex	1	13.5	13.7	13.6	14.9	0.91	0.92	0.91
	2	12.9			14.9	0.87		
	3	12			14.9	0.81		
	4	10.8			14.9	0.72		
	5	9.3	9.3	9.4	14.9	0.62	0.62	0.63
	6	8.5			14.9	0.57		
	7	7.9			14.9	0.53		
	8	0			14.9	0		
100% EtOAc	1	12.4	12.5	12.5	14.8	0.84	0.84	0.84
	2	11.1			14.8	0.75		
	3	10.9			14.8	0.74		
	4	10.5			14.8	0.71		
	5	9.7			14.8	0.66		
	6	7.3	7.2	7.3	14.8	0.49	0.49	0.49
	7	5.1			14.8	0.34		

Continue……

	8	4.1			14.8	0.28		
	9	1.4			14.8	0.09		
	10	0			14.8	0		
1%MeOH/EtOAc	1	12	11.9	11.9	14.8	0.81	0.80	0.80
	2	10.2			14.8	0.69		
	3	9.8			14.8	0.66		
	4	9.1			14.8	0.61		
	5	6.7	6.7	6.6	14.8	0.45	0.45	0.45
	6	4.9			14.8	0.33		
	7	3.8			14.8	0.26		
5%MeOH/EtOAc	1	12.2	12.4	12.4	14.7	0.83	0.84	0.84
	2	10.7			14.7	0.73		
	3	10.2			14.7	0.69		
	4	9.6			14.7	0.65		
	5	8.6			14.7	0.59		
	6	6.9	6.9	6.9	14.7	0.47	0.47	0.47
	7	4.8			14.7	0.33		
	8	3.7			14.7	0.25		
	9	0			14.7	0		
10%MeOH/EtOAc	1	12.2	12.4	12.4	14.8	0.82	0.84	0.84
	2	11.2			14.8	0.76		
	3	10.6			14.8	0.72		
	4	10			14.8	0.68		
	5	9.6			14.8	0.65		
	6	6.9	6.9	6.9	14.8	0.47	0.47	0.47
	7	4.5			14.8	0.30		
	8	3.6			14.8	0.24		
	9	0			14.8	0		
30%MeOH/EtOAc	1	12.2	12	12	14.7	0.83	0.82	0.82
	2		10.4		14.7		0.71	
	3		9.7		14.7		0.66	
	4		8.9		14.7		0.61	
	5	5.6	5.6	5.5	14.7	0.38	0.38	0.37
	6		3.8		14.7		0.26	
50%MeOH/EtOAc	1	12.9	12.6	12.7	14.9	0.87	0.85	0.85
	2	11.7			14.9	0.79		
	3	10.5			14.9	0.70		
	4	9.8			14.9	0.66		
	5	8.9			14.9	0.60		

Continue……

	6	8.1			14.9	0.54		
	7	6.6			14.9	0.44		
	8	4.9	4.7	4.7	14.9	0.33	0.32	0.32
	9	2.4			14.9	0.16		
	10	1.8			14.9	0.12		
	11	0			14.9	0		
75%MeOH/EtOAc	1	12.3	12.3	12.3	14.8	0.83	0.83	0.83
	2	11.2			14.8	0.76		
	3	10			14.8	0.68		
	4	9.1			14.8	0.61		
	5	8.3			14.8	0.56		
	6	7			14.8	0.47		
	7	5.9			14.8	0.40		
	8	4.5			14.8	0.30		
	9	3.9	3.8	3.8	14.8	0.26	0.26	0.26
	10	1.3			14.8	0.09		
	11	0			14.8	0		
100%MeOH	1	12.4	12.4	12.4	15	0.826667	0.826667	0.826667
	2	10.9			15	0.726667		
	3	9.1			15	0.606667		
	4	8.2			15	0.546667		
	5	7.7			15	0.513333		
	6	7.1			15	0.473333		
	7	5.8			15	0.386667		
	8	5.4			15	0.36		
	9	2.7	2.7	2.7	15	0.18	0.18	0.18
	10	0.6			15	0.04		
	11	0			15	0		

DISCUSSION:

In Vitro Anti-Inflammatory Bioassay (Inhibition of Bovine Serum Albumin (BSA) Denaturation Assay)

Hexane, chloroform, ethylacetate, acetone and methanol extracts of *Sinomenium acutum* rhizomes extracts were studies for in vitro anti-inflammatory activity by protein denaturation assay (Table 1). Results obtained from the study demonstrate that extracts HE, CE, EE, AE and ME inhibited protein denaturation and in concentration dependent manner ranging from 5-100 µg/mL except in case of hexane and chloroform extracts that were exhibit non significant percentage of inhibition (<10%) at different concentrations. At maximum concentration i.e. at 100 µg/mL of each of HE, CE, EE, AE and ME extracts produced inhibition at 13.01, 28.54, 60.44, 85.04, 79.21% respectively and keeping the good agreement in comparison with the positive standard Naproxen at equal test concentrations exhibit 89.11% inhibition of protein denaturation.

In Vitro Antidiabetic Bioassay (Glucose Uptake by Yeast Cells Bioassay)

Hexane, chloroform, ethylacetate, acetone and methanol extracts of *Sinomenium acutum* rhizomes extracts were studies for in vitro antidiabetic activity by glucose uptake by yeast cells bioassay (Table 2 & 3). Results obtained from the study demonstrate that extracts EE, AE and ME enhanced glucose uptake by yeast cells and in concentration dependent manner. However, HE and CE are not bioactive fractions as evidenced from the data and no significant percentage enhancement of glucose uptake by yeast cells was displayed in all the test concentrations. Among the tested concentration of the extracts, EE and ME were exhibit nonsignificant percentage of enhancement in glucose uptake by yeast cells (<10%) at

concentrations 5 and 10 µg/mL, while acetone has showed nonsignificant value only at concentration 5 µg/mL. At maximum concentration i.e. at 100 µg/mL of each of EE, AE and ME extracts produced percentage enhancement of glucose uptake at 25.07, 63.19 and 48.21 respectively and keeping with the good agreement in comparison with the positive standard Metformin HCl at equal test concentration exhibit 74.86% enhancement in glucose uptake by yeast cells.

Discussion on Phytochemical Screening

With respect to the phytochemical screening of *Sinomenium acutum* rhizome bioactive extracts (EE, AE and ME) revealed, EE, AE and ME extracts consisting of alkaloids, glycosides, phenols and carbohydrates respectively (Table 4). The bioactive EE, AE and ME extracts respectively answered negative to other screening tests performed for identification of phytochemicals such as flavonoids, proteins, saponins, steroids, tannins and terpenoids, respectively. Consequently, these bioactive extracts were further subjected for TLC observation, so as to determine the phytochemical composition of the bioactive extracts.

Discussion on TLC Observation

TLC technique for separation of bioactive phytochemicals from the bioactive extracts was achieved by using pre-coated TLC plates in different mobile phase concentrations revealed the maximum number of compounds present are up to 13, 13 and 11 Phytochemicals present in each of the bioactive ethylacetate, acetone and methanol extracts respectively of *Sinomenium acutum* with well defined retention factors of each compound present as exposed under UV at 254nm in mobile phase (100% methanol). The optimized mobile phase solvent system for the bioactive extracts to elute maximum number of compounds with good separation factor is 50% methanol/ethylacetate (Figure 1 and Tables 5 to 7).

CONCLUSION:

Based on the present investigation on *Sinomenium acutum* rhizome extracts it can be concluded that among all the extracts tested for their in vitro anti-inflammatory and antidiabetic potential, acetone>methanol>ethylacetate extracts of *Sinomenium acutum* rhizome possess in-vitro anti-inflammatory and antidiabetic activities which might be attributed to the presence of different phytochemicals present in their respective extracts as evidenced from the phytochemical screening and TLC observation in various solvent systems of individual bioactive extract. Protein denaturation activity of the extract could be responsible for the anti-inflammatory activity and likewise improvement in glucose uptake by yeast cells could be remarkable for the antidiabetic activity.

However, further investigation is essential to isolate the bioactive phytochemicals present in individual extract and also to investigate exact underlying mechanism of action these compounds for more specificity towards observed anti-inflammatory and antidiabetic activities.

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