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COMPARATIVE STUDY ON ANTI-MICROBIAL, HYPOLIPIDEMIC AND HYPOGLYCEMIC ACTIVITY OF *AZADIRACHTA INDICA* DIFFERENT FRACTION

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ABSTRACT

The active antioxidant fractions were isolate and identify by disc diffusion method against hazardous microbe *Staphylococcus aureus.* The water, ethanol, methanol, acetone, hexane and butanol solvent were used for the extraction and fractionalization. Antimicrobial susceptibility test showed that different fraction inhibited the growth of *Staphylococcus aureus* (0.4 – 6 mm respectively). The ethanol with methanol fraction highly inhibited the growth of *Staphylococcus aureus* individually. So, these two solvent was once again test new *Staphylococcus aureus* at different concentration. However, this 2:18 (6 mm zone of inhibition) and 10:10 (4mm zone of inhibition) concentrations were highly inhibit *Staphylococcus aureus*. Hence these two fractions were tested against hyperglyceamic and hyperlipideamic in alloxan induced diabetic rats. Among these two fractions the 2:18 concentration of ethanol mingled methanol fraction possess significant antidiabetic activitywhich is as potent as standard antimicrobial and anti diabetic drugs.

INTRODUCTION

Herbal medicines are chief resource for a large majority of people treating health problems (Bharati and Sahu, 2012). It is greatly relied upon especially by rural dwellers, for the treatment of various ailments: traditional doctors or healers are the dispensers of such concoctions (Nuhu and Aliyu, 2008). *Azadirachta indica* belonging to the family of Meliaceae which is universally known by its common English name Neem, have a long history of use for dietary and medicinal purposes throughout the tropics. It is well known in India and its neighbouring countries for more than 2000 years as one of the most versatile medicinal plants having a wide spectrum of biological activity, ayurveda, unani and homoeopathic medicine and has become a cynosure of modern medicine (Biswas et al., 2002).

Azadirachta indica has several medicinal uses such as antigingivitis, antipalque (Chatterje et al., 2012), antioxidant (Shrivastava et al., 2012), antiangiogenic (Mahapatra et al., 2012), chemopreventive (Bharati et al., 2012), larvicidal (Anjali et al., 2012), ovicidal (Mehlhorn et al., 2011), anti-inflammatory, pro-apoptotic, and anti-proliferative (Schumacher et al., 2011) effects. However, standard fraction of an active extract from plant may prove better therapeutically than the crude extract, less toxic and inexpensive compared to pure isolated compounds. But identify the bio-active fraction technique can be complex, costly and time consuming. The aim of our research was to examine different solvent fraction from *Azadirachta indica* against multiple resistance microbes and to study the hypoglycaemic and hypolipideamic activity of *Azadirachta indica* selective fraction in alloxan induced diabetic rat.

MATERIALS AND METHODS

Plant Materials

The fresh leaves of *Azadirachta indica* were collected from Pachamalai Hills, Tamilnadu, India. A voucher specimen of the plant has been deposited at the Department herbarium at Vels University, Chennai, Tamil Nadu, India. Collected leaves were out to obtain only the fresh leaves and washed with distilled water to remove dust particles. After, shade dried leaves were cut into small pieces and powdered using an electric mill.

Extraction

500g of finely ground powder of *Azadirachta indica* were extracted in an all glass Soxhlet extractor for 8 h using methanol as solvents. The extract was dried using a rotary vacuum evaporator for remove solvent and stored in a desiccators until further use

Fractionation

The extract obtained by Soxhlet extraction technique was subjected to fractionation on silica gel. A 250 g of activated silica gel was loaded to a column and cleaned with about 100 ml of hexane (HPLC grade). After, the crude extract of *Azadirachta indica* was loaded on to the column. The compounds were eluted successively with water, ethanol, methanol, acetone, hexane and butanol solvent, respectively.

Micro organisms

The hazardous multi resistance *Staphylococcus aureus* bacterial strain was used in this test which was clinically obtained from clinical patients at dental clinics in and around Thanjavur and Chennai, Tamil Nadu, India.

Determination of antimicrobial activity

In disc diffusion method various fraction of *Azadirachta indica* were tested against hazardous multiple resistance *Staphylococcus aureus* in different petri dish. Strains were swabbed on the surface of the sabouraud agar plates and discs (Whatman No.1 filter paper with 9 mm diameter) impregnated with the 50 µl of each plant sample was place on the surface individually. To compare the anti-bacterial activities, Nystatin (20 µg/disc) used as standard antibiotic and negative control, a blank disc impregnated with solvent followed by drying was used. The plates (triplicates) were incubated 28°C for 72 h. The antimicrobial potency of the test samples was measured by determining the diameter of the zones of inhibition in millimeter.

Animal

Albino rats of either sex weighing between 150-200gm were selected and maintained under ambient temperature (23 ± 2°C), relative humidity (50-55%) with 12-hr light/dark conditions. The experimental albino rats were taken free access of food pellets (Chakan Oil Mills, Sangli, India) and water at ad libitum. After initial determination of 12 h fasting blood glucose levels animals were given single i.p. injection of alloxan at dose 150 mg/kg. The rats were checked for fasting glucose level 72 hours after injection of alloxan, and those with blood glucose greater than 200 mg/dL were considered as diabetic rats**.**

Experimental procedure

The diabetic rats were divided into following groups of six animals each:

Group I : Served as control (Normal saline)

Group II : Served as diabetic control (Alloxan alone)

Group III : Diabetic rats treated with ethanol combine methanol fraction (10:10)

Group V : Diabetic rats treated with ethanol combine methanol fraction (2:18)

Group VI: Diabetic rats treated with Glibenclamide (5mg/kg/day)

All treatment with test drugs and standard were given orally and continued for 28 days, respectively. Blood samples were collected from rats at 7 days interval by cardiac puncture under anesthesia and centrifuging at 4000rpm for 10min to obtain clear serum. The serum glucose was estimated by GOD/POD method (Trinder, 1969), whereas serum TC, TG, (McGowan et al., 1983) HDL, LDL and VLDL (Allain, 1974) were measured by standard method.

Statistical analysis

The data were statistically analyzed and all values were expressed as mean ± S.E.M. The data were also analyzed by one way ANOVA followed by Dunnet's t-test.[12] $P<0.05$ was considered significant.

RESULT AND DISCUSSION

Diabetes mellitus is chiefly characterized by chronic hyperglycemia, with disturbances of carbohydrate, lipid and protein metabolism resulting from defects in insulin secretion, insulin action or both (Koti et al., 2011). Because, oxidative stress resulting from enhanced free radical formation or defects in antioxidants defence causes severe tissue damage and may lead to number of diseases like coronary artery disease, atherosclerosis, cancer and diabetes (Chakraborty and Das, 2010). Plant materials which are being used as traditional medicine for the treatment of diabetes are considered one of the good sources for a new drug or a lead to make a new drug (Umashanker and Shruti, 2011). The active fraction from the effective extract is important in the development of novel drugs (Jarald et al., 2009).

Present study, *Azadirachta indica* water, ethanol, methanol, acetone, hexane and butanol crude extract and fraction individually showed normal anti-bacterial activity. The average zone of 2, 5, 6, 0.4, 0.8, 2 mm zone inhibitions of fractions were analyzed (Table 1). The observed active fraction of ethanol and methanol were further combining various concentrations and over again treated with novel *Staphylococcus aureus* strain (Table 2). The 2:18 (6 mm zone of inhibition), 10:10 (4 mm zone of inhibition) combinations of ethanol with methanol fraction showed the highest activity against the growth of *Staphylococcus aureus.* Hence the selective antimicrobials effects of these two fractions were selective for anti-diabetic study.

Table 1: Antimicrobial activity of *Azadirachta indica* individual fraction tested against *Staphylococcus aureus* by disk diffusion method.

Plant sample				Zone of inhibition (mm)					
Concentration	**18:2**	**16:4**	**14:6**	**12:8**	**10:10**	**8:12**	**6:14**	**4:16**	**2:18**
Azadirachta indica	**(E/M)**	**(E/M)**	**(E/M)**	**(E/M)**	**(E/M)**	**(E/M)**	**(E/M)**	**(E/M)**	**(E/M)**
	0.3	0.6	1	-	4	1	1	-	6

Table 2: Antimicrobial activity of ethanol and methanol combined fractions of the *Azadirachta indica* tested against *Staphylococcus aureus* by disk diffusion method.

Plant sample / Solvent	Zone of inhibition (mm)					
	Water	**Ethanol**	**Methanol**	**Acetone**	**Hexane**	**Butanol**
Azadirachta indica	2	5	6	0.4	0.8	2

Table 3: Effect of selective fraction of *Azadirachta indica* leaves on the serum lipid profile of normal, diabetic induced and drug treated adult albino rats.

Parameter	Serum Glucose (mg/dl)	TC (mg/dl)	TG (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)
Group I (Normal control)	99.07±1.13	116.12±12.43	78.12±7.84	73.66±8.12	21.41±1.45	51.06±5.12
Group II (Diabetic control)	278.03±11.21	277.13±17.31	129.13±12.15	203.12±13.65	34.87±2.51	21.14±7.06
Group III (Ethanol: Methanol fraction-10:10)	135.21±12.33*	124.07±19.01*	96.23±5.61*	85.62±8.14*	23.12±4.12*	31.15±6.12*
Group IV (Ethanol: Methanol fraction-2:18)	128.12±14.52**	111.12±11.45**	86.13±3.16**	61.12±5.17**	18.03±6.17**	35.14±5.12**
Group V Glibenclamide (5mg/kg)	110.07±13.61**	90.17±8.91**	81.04±4.56**	57.07±6.16**	16.26±3.01**	40.28±7.08**

Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one way ANOVA followed by Dunnet's test. *P<0.05,

In anti-diabetic activity, alloxan increase the blood glucose levels in the range of 265-291mg/dl. In Table 3, we found that the 2:18 and 10:10 combination of ethanol with methanol fraction of *Azadirachta indica* treated and standard drug glibenclamide (5mg/kg) treated groups (Groups III,

IV and V) was found to reduce the decrease of serum glucose and plasma lipid levels in diabetic rats on 28th day, respectively. The significant percentage reduction on TC, TG, LDL, VLDL, and increased HDL in 2:18 combination fraction treated group (Group IV) then 10:10 combination treated group (Group III). This revealed that *Azadirachta indica* leaves 2:18 ethanol methanol fraction have higher activity than same solvent 10:10 concentration. From this study, we can conclusively state that the higher zone of inhibition of active fraction have also been reduce the diabetic risk because of antimicrobial drug from plants have posses significant free radical scavenging activity. On the whole, this paper aims at reporting disk diffusion method is the easiest and low cost way to identify active antioxidant drug to treat diabetic complexions.

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