

## Biological Activity of *Anisotes trisulcus* Extract as Antioxidant and its Effect on Blood Sugar Level

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(Received: 03 September 2014; accepted: 09 November 2014)

Nowadays medicinal plants characterized by great stature where it is one of the main resources of medicinal drugs. The study chooses the *Anisotes trisulcus* as a platoon of alakancih as medicinal plant to conduct the studies of this research on chemical analysis and the effect of plant extracts as antibacterial and antifungal after extraction by cascade separation by several solvents, then study the effects of this plant on sugar level in the blood by giving the plant as a medicine to experimental mice have been struck with diabetes, and detect the effect of plant extract on group of other blood characteristic. The extraction process conducted on the plant and the process of separation and distinguishing the plant component by GC.MS instrument. By studying the antioxidant effect of plant extract, the stem extract has the biggest inhibition effect on free radicals, followed by leaves then roots extract. As for the effect of aqueous extract of the plant on level of sugar in blood, the experiments which were held on diabetic experimental mice show that the effect was clear by lowering sugar level from 267.3 to 141.39 mg/dl, which means important indication for validity of this extract as treatment of high levels of sugar in blood. At the same time, the effect of this extract led to controlling many of diabetic experimental mice blood characteristics and returned it to near numbers of the normal level like white blood cells, Hemoglobin concentration, number of platelets, and percentage of granulocytes.

**Key words:** *Anisotes trisulcus*; Diabetes; Antioxidant; Blood Sugar.

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The differences in geography of Saudi Arabia support plant diversity of local, national, and international medicinal plant. There are few hundreds of valuable plants in fold medicine and most of these plants under study now or has not been studied completely as medicinal value. Also the scientific progression in the modern era increased the understanding of medicinal plants with the help of a lot of available reports and

documents about the plants (stockwell, 1988). The scientific research still holds to us plenty of harmful side effects of some of manufactured medicines either because low knowledge of these drugs or because it is a concentrated chemical material prepared in the laboratory under hard chemical reactions (Bhuraneswari *et al.*, 2002).

Therefore, we are finding now a big necessity pouching us toward safe alternatives to resist and combating pathogenic bacterial agents without exposure to its harmful side effects, one of these examples the usage of plant extracts of

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medicinal and aromatic plants which have effective impact on destroying harmful bacterial species, and due to the differences in the chemical structure of these compounds, the components of plants extract differ in their characteristics and effects on other organisms (Bruchrer, 1999).

*Anisotes trisulcus* one of these plants which is spreading in the south of Saudi Arabia in Jazan area and in narrow scope at Baha area (Collenette, 1985), this plant was used in folk medicine to treat liver diseases and known in his ability to lower Blood Pressure (El-shanawany *et al.*, 2011; Fleurentin *et al.*, 1986), a study conducted on this plant showed his effect as appetit suppressant which make it suitable drug for weight loss for obese patients (Al-Rehaily *et al.* 2010). Another study also conducted by (Al-Rehaily *et al.* 2002) this study identify some new alkaloid materials which separated from aerobic parts of plant, *Anisotes trisulcus* was selected by this research as medicinal plant to study some of the biological activity of extracts of its stem and leaves by different solvents.

The study aim to Extract the active materials of *Anisotes trisulcus* by different solvent and identify its chemical component, then study the antioxidant effect of plant extracts and test the efficacy of these extracts against diabetes and its effect on blood characteristics of experimental mice.

## MATERIALS AND METHODS

### Collection of samples

*Anisotes trisulcus* collected from Jazan area at spring 2012. Weighing 100 g of dry samples and put in 1000ml Flask, then a 200 ml of 95% ethanol was added to the flask (Nwosuokafor, 1995) then steamed to get the active ingredients in solid phase. The solid materials befell by mixture of ethanol and water in percent 40%-60%, followed by cascade separation by alcoholic solvents Petroleum ether, chloroform, Ethyl acetate, n-butanol respectively (grand *et al.*, 1988).

### *Anisotes trisulcus* extract chemical analysis

*Anisotes trisulcus* extracts samples of stem and leaves prepared by different solvents with cascade separation and injected to GC.MS to identify the different active ingredients of plant extract.

### Estimate the antioxidant efficacy

The estimation of extract antioxidant efficacy by antioxidant activity test DPPH (Molyneux, 2004), this test depend on inhibition of free radicals, where we leave the extract to react with DPPH for 30 minutes which turn to DPPH-H, with the knowledge that free radicals are relatively stable with loss of absorbance high wavelength  $\lambda_{max} = 517$  nm, then calculating the percentage of inhibition by role  $I\% = (A_0 - A_i) / A_0 \times 100$  (Roy *et al.*, 2010). Whereas  $A_0$  light absorbance to free radicals without presence of extracts,  $A_i$ : light absorbance for mixture (extracts+ radicals).

### Effect of *Anisotes trisulcus* extract on the level of sugar in blood

30 male mice gutted from animal house at Pharmacy College in King Saud University, Saud Arabia. 20 mice were injected under peritoneum with single dose of Streptozotocin (SIGMA, USA) by 65mg/kg to produce diabetic mice (Pari and Murugan, 2007). After 72 hours of Steptozotocin injection, a blood sample from mice tail vein was collected to detect the level of sugar in blood. The diabetic mice divided into two groups. First diabetic group was given the water extract of *Anisotes trisulcus* leaves and stems by 100mg/kg dose for 28 days, while the second diabetic group was given distilled water for 28days (N), as control group (C), 10 mice given phosphate buffer.

### Measurements of sugar levels in blood, body weight, food and water consumption

Sugar level in blood and body weight was measured weekly after night fasting for 12 hours, fasting sugar was measured by sugar instrument at (Science College in King Saud University, Saudi Arabia) at fixed time. While blood collection: was from eye corner of mice by capillary tube containing heparin to make complete blood count (Waynforth, 1980).

### Statistical analysis

All statistics done by SPSS V19 identify the presence of significance differences in the results.

## RESULTS AND DISCUSSION

### *Anisotes trisulcus* chemical analysis

Table 1 illustrate that the extract of plant contain many compounds, the most important one is preganine on 5.42 time, this result is consistent

with finding of (Al-Rehaily *et al.*, 2002) in his study on the extract of *Anisotes trisulcus*. It also contains 7-hydroxy vasicine on time 4.35, and 5-methoxypeganine on time 6.50 and this consistent with study of (El-Shanawany *et al.*, 2011) on the extract of *Anisotes trisulcus*.

It also appeared during the analysis some component did not appear in any previous study conducted on this plant like Pentadecanal on time 15.32, and Undecanoic acid ethylester on time 10.26, this is may be due to differences in geogaphical and climate of the region where the plant collected which conflict on the type and percentage of it is active ingredient content.

#### Estimation of antioxidant efficacy

The antioxidant effect of *Anisotes trisulcus* as in Table 2, it shows that plant stem extract was the most effective as antioxidant from other parts of plant, where the percentage of free radical inhibition at the minimum concentration 32.12%, it shows also 51.39% inhibition at 4mg/ml concentration, the highest level of inhibition was at 8mg/ml with 70.28%, these results consistent with finding of (Jimenez *et al.*, 2009) at his study on the effect of *Justicia spicigera* leaves extract as antioxidant, which belong to the same species of *Anisotes trisulcus*.

Followed by leaves extract where the antioxidant percentage reach 27.96% at concentration 2mg/ml, it raised gradually to 46.85% at concentration 4mg/ml, then reached to its highest level with 63.85% at concentration 8mg/ml, this is consistent with study of (Ahmad *et al.*, 2012) at his study on *Ruellia tuberosa* L leaves as antioxidants which belong to the same species of *Anisotes trisulcus*. Regarding the extract of roots, it was the least efficacy in the inhibition of free radicals, where the inhibition percentage didn't

exceed 11.3% at concentration 2mg/ml, and graduated until 18.5% at concentration 4mg/ml, and didn't exceed 20.6% at the highest used concentration 8mg/ml.

Regarding plant content of vitamin C, and A, leaves was contain the highest percentage of vitamin A by 1614.99mg/100g, which mean 20 times more than the content of roots from this vitamin. While the leaves contains 107.89 mg/100g of vitamin C, followed by stem with 87.49/ 100g, followed by roots which have the least percentage with 18.4mg/100g.

From the effects and results its appear that *Anisotes trisulcus* plant with his different parts specially the stem possesses good effectiveness as antioxidant, and this efficacy may be due to containment of the vitamins mentioned above.

#### Effect of *Anisotes trisulcus* plant on blood sugar level

Regarding to efficacy of *Anisotes trisulcus* leaves and stem extracts on blood characteristics of Diabetic experimental mice, it

**Table 1.** Shows components of *Anisotes trisulcus* during injection of plant extract in GC.MS about 12 compounds

No	Compound name	Retention time
1	7-hydroxy vasicine	4.38
2	peganine	5.42
3	Methyl tetradecanoate	6.50
4	anisotine	7.34
5	Hexadecanoic acid 15-methylester	8.11
6	7-hydroxy vasicinone	9.55
7	5-methoxypeganine	12.11
8	Hydroxylamine.O-decyl	13.17
9	Undecanoicacid.ethylester	13.57
10	trisulcusine	14.15
11	Pentadecanal	15.32
12	Dodecanal	10.26

**Table 2.** Shows the percentage of inhibition the root, stem and leaves extracts from *Anisotes trisulcus* plant as antioxidant with the contents of these parts of the vitamins A, C

Biochemical Constituents	<i>Anisotes trisulcus</i>			
	Root	Stem	Leaves	
Vitamin A "µg/100g"	66.2 ± 5.9	1115.74 ± 102.5	1614.99 ± 136.1	
Vitamin C "mg/100g"	18.4 ± 2.9	87.48 ± 6.4	107.89 ± 6.8	
Antioxidant Activity (%)	25 µl(contain 50 µg of extract)	11.3 ± 1.5	32.12 ± 3.7	27.96 ± 3.7
	25 µl(contain 100 µg of extract)	18.5 ± 2.7	51.39 ± 4.5	46.85 ± 4.6
	25 µl(contain 200 µg of extract)	20.6 ± 2.2	70.28 ± 5.2	63.85 ± 3.9

turns out that the efficacy of these extracts very huge through big percentage of efficacy of controlling most characteristics and quality of blood which the study was conducted on, as it is shown on table (3), the most important one was reducing sugar level from 267.3 to 141.39 mg/dl and this is consistent with the finding of (Shahwara *et al.*, 2011) in his study on the efficacy of *Ruelliatuberosa* on blood sugar levels follow the same species which the *Anisotes trisulcus* belong to, where it reduce the blood sugar level with 15%, but it was much less than the percentage we get it in this study which reach 53%, which consider as important sign for validity of *Anisotes trisulcus*

extract usage as treatment for high blood sugar, the extract also lower the big inflation of RBCs number to half of its level, this is mean that control with 50% percentage, the same thing with inflation of lymphocytes, monocytes, and Hemoglobin concentration.

Regarding the number of WBCs number, size of RBCs, and percentage of granulocytes, and number of platelets, it has been returned to levels near to its normal levels in blood. And this indicates the extent of the influence the extract on repairing the imbalance in blood characteristics due to the elevation in blood sugar and the possibility of usage as treatment for these things.

**Table 3.** Illustrates the special qualities of the blood of mice measurements are divided into the first three groups of infected diabetic and treatment extracted water for the stems and leaves of the plant *Anisotes trisulcus* infected diabetic and non-treatment of Dia and the second normal Control and third

Parameter	<i>Anisotes trisulcus</i>		Dia		Control		Range	Unite
	Mean	Std. Error	Mean	Std. Error	Mean	Std. Error		
Sugar after 3 days	207.62	4.6241	226.2	16.535	134.45	2.98	120 - 140	gm/dl
Sugar after 31 days	141.39	8.4253	267.3333	11.128	132.45	3.526		
WBC	10.63	0.3152	94.2	4.3501	5.24	0.3592	0.8 - 6.8	10 <sup>9</sup> /L
Lymph.	7.16	0.2124	10.0667	1.4518	4.258	0.2458	0.7 - 5.7	10 <sup>9</sup> /L
Mon.	0.41	0.0758	2.8667	0.3844	0.285	0.0125	0.0 - 0.3	10 <sup>9</sup> /L
Gran.	2.34	0.9954	97.9333	2.8292	1.589	0.0985	0.1 - 1.8	10 <sup>9</sup> /L
Lymph%	67.26	9.5365	9.5667	0.6333	78.569	2.0365	55.8 - 90.6	%
Mon%	4.10	0.8523	2.7333	0.1453	5.7	0.1258	1.8 - 6.0	%
Gran%	28.53	3.358	87.7	0.7767	28.562	0.3568	8.6 - 38.9	%
RBC	7.86	1.4253	8.4467	0.1532	6.425	0.1125	6.36 - 9.42	10 <sup>12</sup> /L
HGB	10.33	0.4856	28.2333	2.4443	13.2	0.9562	11.0 - 14.3	g/dL
HCT	40.16	1.1255	34.8333	2.2259	44.425	1.5236	34.6 - 44.6	%
MCV	50.94	1.8952	42.1667	2.3412	55.125	1.6582	48.2 - 58.3	fL
MCH	13.00	0.7851	33.3333	2.5983	17.258	0.8562	15.8 - 19.0	pg
MCHC	25.56	0.2586	79.0333	1.8478	32.586	0.1458	30.2 - 35.3	g/dL
RDW	19.21	0.1345	20.8	2.2546	14.256	1.8562	13.0 - 17.0	%
PLT	604.86	22.9658	994	28.8675	394	18.2586	100 - 600	10 <sup>9</sup> /L
MPV	6.21	0.3654	5.8667	0.2603	6.954	0.2245	5.5 - 7.5	fL
PDW	15.04	0.3356	14.6667	0.3712	16.552	0.3215	-	-
PCT	0.26	0.0856	0.5103	0.0562	0.268	0.0215	-	%

#### Significance of abbreviations that represents the blood characteristics and qualities

WBC: White Blood Cell, RBC: Red Blood Cells, Hgb: Hemoglobin, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, MPV: Mean Platelet Volume, RDW: Red cell Distribution Width, LY%: Lymphocytes percentage, MO%: Monocytes percentage, GR%:

Granulocytes percentage, LY#: Lymphocytes number.

#### CONCLUSION

1. Isolation of active ingredients that has the activity on lowering blood sugar and use it as treatment of diabetic patients.
2. Benefit from the effectiveness of *Anisotes*

*trisulcus* plant extract as antioxidants in different fields which need this effect.

- Maintaining the growth and spread of *Anisotes trisulcus* plant, and protect him from the danger that raid the plant and introducing it to new areas by rebuilding and maintain vegetation.

#### ACKNOWLEDGEMENTS

This project was supported by the King Saud University, Deanship of Scientific Research, College of Science Research Center.

#### REFERENCES

- Ackacha, M. A. Removal of Zn(II) Ions from Aqueous Solution by New Adsorbent: *Calligonum comosum*. *Int. J. Chem. Environ. Engin.*, 2010; **1**(1):
- Al-Yahya, M.A. Phytochemical studies of the plants used in traditional medicine of Saudi Arabia. *Fitoterapia*, 1986; **57**(3): 179-182.
- Badria, F.A., Ameen, M., Akl, M.R. Evaluation of cytotoxic compounds from *Calligonum comosum* L. growing in Egypt. *Z Naturforsch C*, 2007; **62**(9-10): 656-60.
- El-Hag, E.A, Harraz, F.M., Zaytoon, A., Salama, A. K. Evaluation of some wild herb extracts for control of mosquitoes (Culicidae, Dipetra). *J. King Saud Univ.*, 1996; **8**(1): 135-145.
- El-Hawary, Z.M., Kholief, T.S. Biochemical studies on some hypoglycemic agents (II) effect of *Calligonum comosum* extract. *Archiv. Pharmacol Res.*, 1990; **13**: 113-116.
- Grand, A., Woudergem, P.A., Verportes, R., poussel, J.L. Anti-infection phytotherapies of tree-savannah sengl (West-Africa), II-Anti microbial activity of 33 species. *J. Ethnopharm.*, 1988; **22**: 25-31.
- Karuppusamy, S., Rajasekaran, K.M. A New Approach to High Throughput Antibacterial Screening of Plant Extracts by Resazurin Redox with Special Reference to Medicinal Plants of Western Ghats. *Adv. Biolo. Res.* 2010; **4** (4): 201-206
- Molyneux, P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. Sci. Technol.*, 2004; **26**(2): 211.
- Nwosu, M.O., Okafor, J.J. Preliminary studies of the antifungal activities of some medicinal plants against Basidiobolus and some pathogenic fungi. *Mycoses*, 1995; **38**: 191-195.
- Pari, L., Murugan, P., Tetrahydrocurcumin Prevents Brain Lipid Peroxidation in Streptozotocin-Induced Diabetic Rats. *J. Med. Food*, 2007; **10**: 323-329.
- Riadh, H., Imen, F., Abdelmajid, Z., Sinda F. Detection and extraction of antilisterial compounds from *Calligonum comosum*, a medicinal plant from arid regions of Tunisia. *Afr. J. Tradit Complement Altern Med.*, 2011; **8**(3): 322-327
- Roy, M.K., Koide, M., Rao, T.P., Okubo, T., Ogasawara, Y., Juneja, L.R. ORAC and DPPH assay comparison to assess antioxidant capacity of tea infusions: Relationship between total polyphenol and individual catechin content. *Int. J. Food Sci. Nut.*, 2010; **61**(2): 109.
- Salama, H., Marraiki, M.H.N. Antimicrobial activity and phytochemical analyses of *Polygonum aviculare* L. (Polygonaceae), naturally growing in Egypt. *Saudi J. Biologic. Sci.* 2010; **17**: 57-63
- Shahidi, Bonjer, G.H., Aghighi, S., Karimi, Nik, A. Antibacterial and Antifungal Survey in Plants used in Indigenous Herbal-Medicine of South East Regions of Iran. *J. Biologic. Sci.*, 2004; **4**(3): 405-412.
- Sing, Wadhvani, Singh UR, Wadhvani AM (1996). Dictionary of Economic plants in India. ICAR New Delhi, ISBN: 3768200019, 1996; 37-60.
- Waynforth, H.B. Experimental and Surgical Technique in the Rat. Academic Press Inc., (London) LTD, NWI, 1980; 29.