Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/bkpbvh77

ANTI-INFLAMMATORY, ANTI-DIABETIC AND LARVICIDAL ACTIVITY OF ETHYL ACETATE FRACTION OF LEAVES AND STEM OF *VIBURNUM COTINIFOLIUM* D.DON.

Saima[1], Tanvir Burni[2], Rasool Khan[3], Muhammad Jamal[4], Habib Ullah[5]

[1*,2]Department of Botany, University of Peshawar, Pakistan.
[3]Institute of Chemical Sciences, University of Peshawar, Pakistan.
[4]Department of Pharmacy, City University of Science and Information Technology, Pakistan.
[5] Department of Zoology, University of Peshawar, Pakistan.

[*]**Corresponding Author:** Saima
[*]Department of Botany, University of Peshawar, Pakistan.

ABSTRACT

Vibernum cotinifolium is a deciduous shrub growing up to 3.5 m. The plant is traditionally used for many treatments. *Viburnum cotinifolium* is well known in folk medicine for sedative, spasmolytic, antioxidants, antibacterial, astringent and anti-asthematic properties. In current study the Larvicidal, anti-inflammatory and anti-diabetic activity of the ethyl acetate fraction of stem and leaves of *Vibernum cotinifolium* has been explored. In larvicidal activity the extracts were tested against *Adies* larvae and showed moderate to high toxic effect on *Adies* larvae after 72 hours of exposure at 300ppm concentration, while maximum inhibition (80%) was observed in ethyl acetate fraction of leaves of *V. cotinifolium* with LC_{50} value of 187.5 ppm. In stem ethyl acetate fraction the maximum inhibition was 71.7 percent after 72 hours of exposure at the concentration of 300ppm. In carragenan-induced paw edema model each fraction was supplied at a dose of 100 mg/kg, 200 mg/kg and 300 mg/kg. At 300 mg/kg the ethyl acetate fraction of leaves showed 54.83 % inhibition. While the stem fraction showed 49.82% inhibition. In anti-diabetic activity results were well comparable with the standard drug glibenclamide. The stem fraction was more effective as compared to standard drug with 86.07 percent inhibition. In all the three bioassays the plant showed potent inhibition which ensure its uses as cure for these diseases.

Key words: Larvicidal, anti-inflammatory, anti-diabetic, ethyl acetate.

Introduction

Viburnum cotinifolium is well known in folk medicine for sedative, spasmolytic, antioxidants, antibacterial, astringent and anti-asthematic properties (Alam *et al.,* 2014). *Vibernum cotinifolium* is a deciduous shrub growing up to 3.5 m. The plant is traditionally used for many treatments. Plants are crucial for human survival, providing food, fiber, shelter and medicinal benefits. Many physiologically active compounds, including minerals and phytochemicals, that are present in medicinal plants have a range of effects on people (Dagli *et al.,* 2015). Controlling mosquitoes is an essential public health measure everywhere, but particularly in tropical regions. For lowering the prevalence of diseases carried by mosquitoes, controlling mosquito immatures in their breeding grounds is the most applied strategy. Because of their rapid knockdown effect, synthetic organic pesticides have been employed for many years to control vectors and pests of several human

diseases. However, their careless usage led to a number of issues, including poisonous residues in food, threats to the environment, the eradication of natural adversaries, and the development of insecticidal resistance in important vector species (Macedo *et al*., 1997). The development of novel insecticides that are readily available, reasonably priced, do not harm non-target populations, and degrade readily is imperative in order to address these issues (Ansari *et al.,* 2000). The main reason why plants have been suggested as substitutes for traditional mosquito larvicides is that they may contain bioactive secondary compounds that the general public believes to be reasonably safe, low environmental risk, and having little effect on the health of humans and animals (Shaalan *et al*. 2005). Secondary drugs frequently act on many and novel target locations (Isman, 2006), lowering the likelihood of resistance (Wang *et al.,* 2012). They are considered prospective sources for producing commercial pesticides since certain plant preparations and their constituents fit the criteria for low-risk insecticides (Isman, 2008).

Plants primarily store phytochemicals as secondary metabolites, which act as a conduit for the plants' defensive mechanisms. Previous reports of the insecticidal effects of secondary metabolites from various plants have included alkaloids, steroids, terpenoids, tannins, and flavonoids (Shaalan *et al*., 2005). In order to control insect pests of medical importance, plant products or compounds derived from plants offer a promising substitute for synthetic insecticides. These products are low-cost, biodegradable, environmentally safe, and can be produced using traditional methods for vector control (Haldar *et al.*, 2011). Additionally, they can be used by individuals and communities with little to no care and certain herbal products have been used as natural insecticides in the past, including nicotine made from tobacco leaves, lupinine and anabasine, which are alkaloids extracted from Russian weed *Anabasis aphylla* (Campbell *et al.,* 1933), rotenone from *Derris eliptica* (Zubairi *et al*., 2004), and pyrethrums from *Chrysanthemum cinererifolium* flowers (Hartzell & Wilcoxon, 1941). Ghosh *et al.* (2012) and Kishore *et al.* (2011) have also examined the current state of research on plant extracts' effectiveness in mosquito control studies.

Inflammation is the reaction of tissues and cells of the body to wound due to different aspects such as chemicals, infections and thermal and mechanical injuries (Kaushik *et al.,* 2012). Inflammation can be categorized as chronic and acute inflammation and is a very common health problem. While many drugs are known to treat inflammatory ailments but their persistent use often produce stomach problems, water and salt retention, bone marrow reduction (Matthew *et al.,* 2013). Therefore, finding natural anti-inflammatory medications with minimal adverse effects, especially those derived from plants is essential. Many phytochemicals found in medicinal plants, such as alkaloids, flavonoids, triterpenoid, and saponins, have anti-inflammatory qualities and function via a variety of pathways, such as protease inhibition, albumin denaturation, and COX enzyme inhibition (Fawole *et al.,* 2010; Modi *et al*., 2019). The body's reaction to being harmed by chemical or physical factors is inflammation. Discomfort, redness, swelling, heat, and loss of function are its hallmarks. Numerous synthetic medications, such as aspirin, diclofenac, aceclofenac, and indomethacin, have strong anti-inflammatory properties; However, many of these drugs are either prohibited or used less frequently for long-term treatment due to severe adverse effects include liver damage, kidney failure, and upper gastrointestinal hemorrhage (Patidar *et al.,* 2014).

Diabetes mellitus is an endocrine disease with most prevalence in the world and is the major epidemic in the human history. Many synthetic medicines are made and introduced into market to treat the diabetes, but its long-term use is limited. So there is a need to discover new drugs from natural sources with low side effects (Hasanpour *et al.,* 2020). Diabetes mellitus is a multifaceted illness marked by a severe disruption in the metabolism of proteins, fats, and carbohydrates as a result of insufficient insulin secretion or action (Luo *et al.,* 2004). World Health Organization reports that 88% of the population in 198 nations uses traditional medicines. Many nations have developed policies regarding conventional medicine (WHO, 2019). The main advantages of medicinal plants are their low cost, accessibility, and lack of side effects. Antimicrobial, anti-inflammatory, antidiabetic, and antioxidant qualities have been found for a variety of medicinal plants (Modi *et al.,* 2019; Kaneria *et al.,* 2009; Patel *et al.,* 2019). Therefore, finding natural, long-

lasting anti-inflammatory and anti-diabetic medicines that work without causing negative side effects is of utmost importance.

Materials and method

Plant collection and powder drug formation

The parts of the plant, *Viburnum cotinifolium,* consisting of leaves and stem were collected from upper Dir and subsequently made cleaned, dried, pressed and spread in shade for 15 days for the purpose of drying. Afterwards, an electric grinder was used in order to grind the dried parts of the plant into a fine powder.

Extraction and Fractionation

1000 gram of dried powdered material was macerated in 1500ml of methanol at room temperature for three days then filtered to obtain the crude extract. The water bath at 40°C was used to further evaporate the solvent completely in order to make the extract free of solvent. Three crude methanol extracts were fractionated with the solvent ethyl acetate. The crude extract was dissolved with water amounting to 250ml in a separation funnel followed by vigorous shaking and allowing to be settled. Furthermore, the 250ml of ethyl acetate was mixed with it and shaken properly. The solvents were then allowed to settle and after sometime the two layers were separated to obtain the ethyl acetate fraction.

Larvicidal activity

Larvicidal efficacy of the ethyl acetate of crude fractions of leaves and stem was explored following the method of Warikoo & Kumar (2013) against *Adies* larvae. Five batches of 20 larvae each were collected in 249 milliliters of water with 1.0 milliliter of the concentration of the chosen plant extract. Acetone and polysorbate were used to set up the control. Following a 24-hour exposure period, the number of dead larvae was tallied, and the average of five replicates was used to calculate the mortality %.

Anti-inflammatory activity

The fractions of *Viburnum cotinifolium* were assessed for anti-inflammatory properties using a rat paw edema model caused by carrageenan. This method is widely employed for screening potential anti-inflammatory agents. Swiss albino mice, obtained from certified suppliers, were used for the study. The animals were grouped into five groups, each comprising of five animals, for each fraction. Acute paw edema was induced by injecting 0.1 ml of a 1% carrageenan solution (w/v) into the sub plantar tissue of the right hind paw. Group I served as the test group and was treated with carrageenan, while Group II was administered normal saline and served as the negative control. Group III received a standard anti-inflammatory drug for comparison, and the remaining Groups were treated with 100mg/kg, 200mg/kg and 300mg/kg doses of the extracts of *Viburnum cotinifolium*. The linear circumference of the paw was measured hourly for four hours following carrageenan administration, using a vernier caliper. The formula used for the calculation of Anti-inflammatory activity which is expressed as percentage of inhibition of edema, is as follows:

% Inhibition of Edema = $(T-T_0/T) \times 100$

T: Thickness of paw in control group;

T_0: Thickness of paw edema in the test compound treated group.

Antidiabetic activity

In order to evaluate the anti-diabetic activity of the fractions, an alloxan-induced diabetic model in Albino mice was used. For each fraction the animals were grouped into five groups by keeping five mice in each group. Before the experiment, the rats were fasted for 16 hours with water provided ad libitum. Diabetes was induced by administering alloxan, and the blood glucose levels were measured 24 hours after administration using a validated digital glucometer. The groups were

treated as follows: one group served as the solvent control, receiving distilled water orally, another group served as the standard control, receiving a known anti-diabetic drug, and the remaining groups were administered with 100mg/kg, 200mg/kg and 300mg/kg doses of the extracts of leaves and stem of *Viburnum cotinifolium*. Blood samples were collected from the tip of the tail, and glucose levels were determined after each hour for 6 Hours. The gross structure was studied to identify the toxicity effects of plant extracts by dissecting the animals.

Statistical analysis

One way ANOVA was applied to the anti-inflammatory and anti-diabetic data at 5 % level of significance ($p<0.05$).

Results

Ethyl acetate crude fraction of stem and leaves of *V.cotinifolium* were tested against *Adies* larvae and showed moderate to high toxic effect on *Adies* larvae after 72 hours of exposure at 300ppm concentration, while maximum inhibition (80%) was observed in ethyl acetate fraction of leaves of *V. cotinifolium* with LC_{50} value of 187.5 ppm. In stem ethyl acetate fraction the maximum inhibition was 71.7 percent after 72 hours of exposure at the concentration of 300ppm (Tab. 05).

Inflammation is the reaction of cells and tissues of the body to wound due to different aspects such as chemicals, infections and thermal and mechanical injuries (Kaushik *et al.,* 2012). The ethyl acetate fractions of stem and leaves of *Viburnum cotinifolium,* when compared to the control at 1st, 2nd and 3rd hour in the carragenan-induced paw edema model using vernier callipers, produced different results. Each portion was supplied at a dose of 100 mg/kg, 200 mg/kg and 300 mg/kg. At 300 mg/kg the ethyl acetate fraction of leaves showed 54.83 % inhibition. While the stem fraction showed 49.82% inhibition, while Indomethacin at a dose of 10 mg/kg prevented carragenan induced paw edema with a percentage inhibition of 10.19%, 26.13%, 45.89%and 49.53% at 30 minutes, 1 hour, 2 hour and 3 hour respectively. From tables 1 & 2, it is obvious that the fractions of both parts showed dose dependency and also showed significant value ($P<0.05$) at different time intervals.

The phytochemicals in plants play a major role to possess medicinal properties. In current study the anti-diabetic potential of the ethyl acetate fractions of stem and leaves of *V.cotinifolium* was studied and the fractions produced a significant anti-diabetic effect after 6 hours at the dose of 300mg/Kg body weight. These effects were well comparable with the standard drug glibenclamide used in the present study. The ethyl acetate fraction of stem was more effective as compared to standard drug with 86.07 percent inhibition as shown in table 3 & 4. The anti-diabetic activity shown by these crude fractions of stem and leaves is of considerable importance and justified its use as anti-diabetic in future with fewer side effects.

Table No. 1: Anti-inflammatory activity of *Viburnum cotinifolium* leaf and stem

S. No.	Treatment	Dose (mg/kg)	Paw edema after drug administration (Mean ± SEM)				
			0 hour	After 30 min	After 1 hour	After 2 hours	After 3 hours
1.	Carragenen	–	7.19±0.5	13.34±0.06	13.7±0.03	13.9±0.05	13.95±0.06
2.	Standard drug (Indomethacin)	10	7.19±0.4	11.98±0.5	10.12±0.7	7.52±0.8	7.04±0.6
3.	LEA	100	8.01±0.3	11.7±0.6	10.3±0.3	8.6±0.3	7±0.5
4.		200	8.03±0.2	11.6±0.3**	10±0.5*	8.3±0.3	6.6±0.3
5.		300	8.01±0.4	11.3±0.6*	9.6±0.3	8.3±0.3	6.3±0.3
6.	SEA	100	7.95±0.5	11.7±0.9	10.3±0.3	9.3±0.3	7.7±0.3
7.		200	8.04±0.2	11.6±0.3**	10.3±0.9*	8.7±0.3	7±0.4
8.		300	7.31±0.6	11±0.6*	10±0.6**	8.6±0.7**	7±0.6

The data of the anti-infllammatory activity of *Viburnum cotinifolium* are reported as mean ± SEM and were analyzed through ANOVA following Dunnett's post-hoc, significant at *P<0.05 and highly significant at **P<0.01.

Table No. 2: Anti-inflammatory activity of *Viburnum cotinifolium* leaf and stem (% Inhibition).

S. No.	Treatment	Dose (mg/kg)	Paw edema after drug administration (% Inhibition)			
			After 30 min	After 1 hour	After 2 hours	After 3 hours
1.	Standard drug (Indomethacin)	10	10.19	26.13	45.89	49.53
2.	LEA	100	12.29	24.81	38.12	49.82
3.		200	13.04	27.01	40.28	52.68
4.		300	15.29	29.92	40.28	54.83
5.	SEA	100	12.29	24.8	33.09	44.8
6.		200	13.04	24.8	37.41	49.82
7.		300	17.54	27	38.13	49.82

Fig No. 1. Anti-inflammatory activity of leaves of *V.cotinifolium*

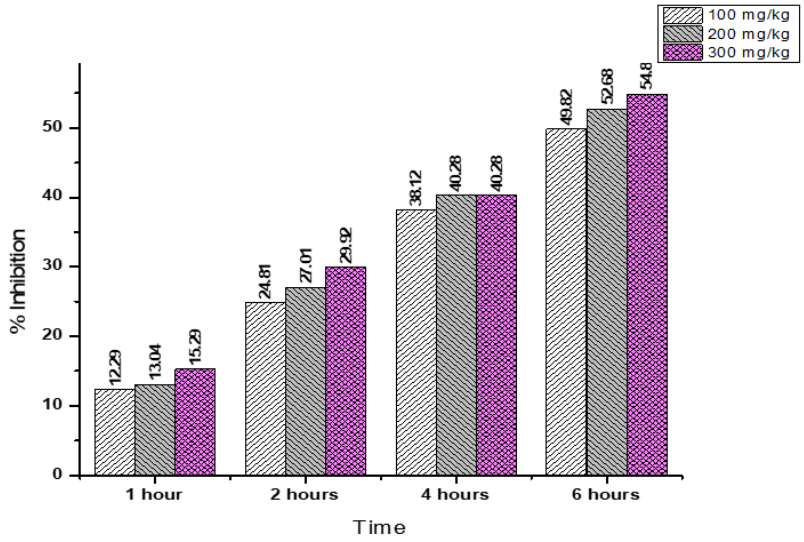

Fig No. 2. Anti-inflammatory activity of stem of *V.cotinifolium*

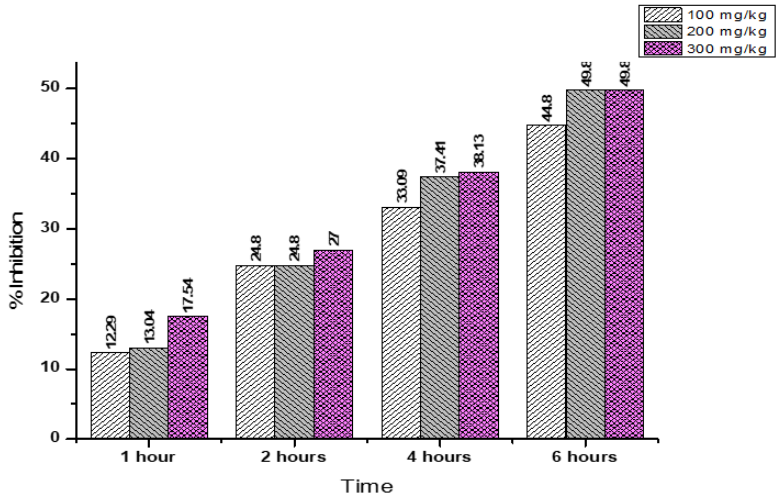

Table No. 3: Antidibetic activity of *Viburnum cotinifolium* leaf and stem.

S. No.	Treatment	Dose (mg/kg)	Glucose level after drug administration (Mean ± SEM)			
			1 hour	2 hours	4 hours	6 hours
1.	Control	-	396±1.9	420±2.2	431±2.1	488±1.9
2.	Standard drug (Glibenclamide)	-	327.5±6.8	247.3±15.5	247.17±13.3	248.3±12.2
3.	LEA	100	323±3.6	258±5.2	199.5±4.5	181±3.4*
4.		200	184±4.4**	167.5±3.4**	138±5.4**	123.5±4.3
5.		300	128.5±3.7**	124±3.5**	112±5.4***	106.5±5.3
6.	SEA	100	374±4.4	332.5±5.3	278.5±3.2*	249±5.6
7.		200	178±3.4**	132±5.3**	126.5±3.4**	103.5±4.5
8.		300	108.5±4.6***	90±5.6**	80±7.2	68±4.2

The data of the anti-inflammatory activity of *Viburnum cotinifolium* are reported as mean ± SEM and were analyzed through ANOVA following Dunnett's post-hoc, significant at *P<0.05 and highly significant at **P<0.01.

Table No. 4: Antidiabetic activity of *Viburnum cotinifolium* leaf and stem (% Inhibition)

S. No.	Treatment	Dose (mg/kg)	Glucose level After drug administration (% inhibition)			
			1 hour	2 hours	4 hours	6 hours
1.	Standard drug (Glibenclamide)	-	17.29	41.12	42.65	49.12
2.	LEA	100	18.43	38.57	53.71	62.91
3.		200	63.53	60.12	67.98	74.69
4.		300	67.55	70.47	74.01	78.18
5.	SEA	100	5.55	20.83	35.38	48.97
6.		200	55.05	68.57	70.65	78.79
7.		300	72.60	78.57	81.44	86.07

Fig No.3. Anti-diabetic activity of leaves of *V.cotinifolium*

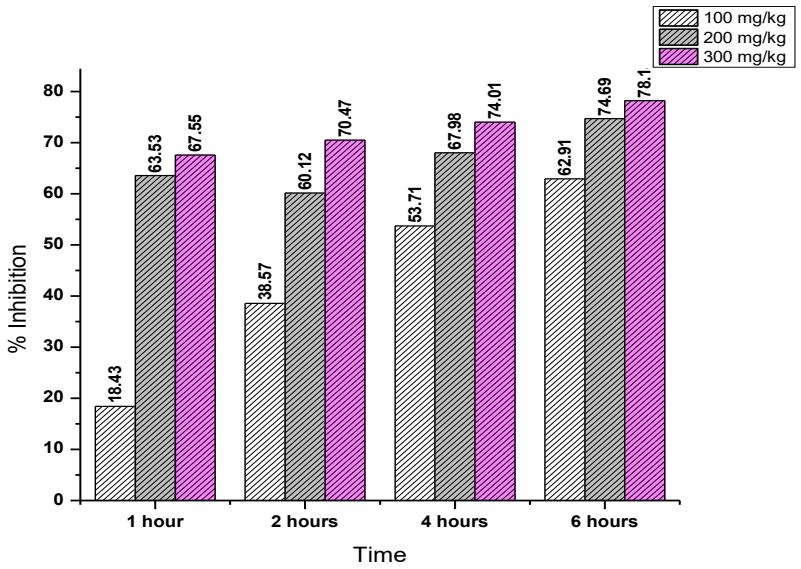

Fig No.4. Anti-diabetic activity of stem of *V.cotinifolium*

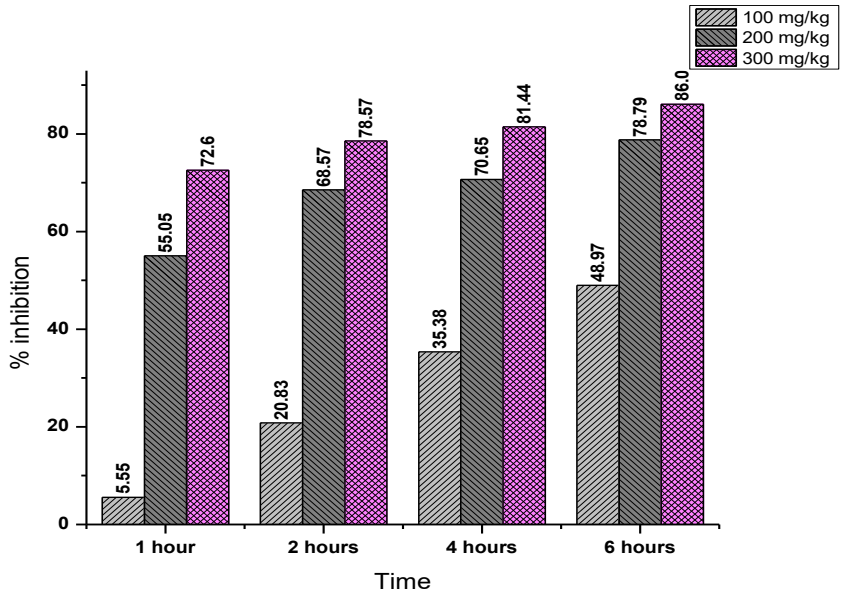

Table No. 5: Larvicidal activity of leaves of *V. cotinifolium.* Values are expressed in terms of % mortality and LC_{50}

Part Used	Time	Concentration used					
		100ppm		200ppm		300ppm	
		% Mortality	LC_{50}	% Mortality	LC_{50}	% Mortality	LC_{50}
Leaves	24 hrs	23.3	214.3	28.3	352.9	30	500
	48 hrs	35	142.9	38.3	260.9	61.7	243.2
	72 hrs	45	111.1	53.3	187.5	80	187.5
Stem	24 hrs	20	250	25	400	26.7	562.5
	48 hrs	35	142.9	36.7	272.7	36.7	409.09
	72 hrs	53.3	93.8	60	166.7	71.7	209.3

Discussion

The main reason why plants have been suggested as substitutes for traditional mosquito larvicides is that they may contain bioactive secondary compounds that the general public believes to be reasonably safe, low environmental risk, and having little effect on the health of humans and animals (Shaalan *et al*. 2005). They are considered prospective sources for producing commercial pesticides since certain plant preparations and their constituents fit the criteria for low-risk insecticides (Isman, 2008). It is believed that the complex mixture of active compounds in plants is responsible for the larvicidal activity. The chemical components in the leaf and stem that stop the metabolic processes of larvae may be the cause of the high larval death rate (80%). Plant extracts can either directly affect the epidermal cells that produce the enzymes for the tanning or cuticular oxidation process, or they can block neurosecretory cells (Jeyabalan & Murugan 1999). Eco-friendly and plant-based insecticides have become more and more popular in recent years. Because of their easy biodegradability and target specificity, they are safe to employ in aquatic environments.

Inflammation is the reaction of cells and tissues of the body to wound due to different aspects such as chemicals, infections and thermal and mechanical injuries (Kaushik *et al.,* 2012). Worldwide, inflammatory diseases have long been treated with immunosuppressive therapies, steroidal medicines, and non-steroidal anti-inflammatory drugs (NSAIDs). The effectiveness of non-steroidal anti-inflammatory medications has been evaluated using the carrageenan-induced rat paw edema model, which is amenable to cyclooxygenase (COX) inhibitors (Rao *et al.,* 2005). According to

Hajhashemi *et al.* (2009), they are associated with severe adverse consequences such peptic ulcers and gastrointestinal bleeding. Many natural medicines originating from plants and marine creatures have recently been regarded useful and safer for the treatment of many disorders including inflammation and pain (Su *et al.,* 2011). The anti-inflammatory impact of extracts is most likely owing to its influence on the second phase of inflammation, the cyclooxygenase pathway rather than the lipoxygenase system. This is validated by the maximum suppression of inflammation at the end of the third hour following the carrageenan challenge (Chaudhari *et al.,* 2012). The phytochemical analysis of stem and leaf ethyl acetate fractions of *V.cotinifolium* indicated the presence of flavonoids, glycosides, saponins, steroids, tannins, and polyphenols. The significant anti-inflammatory action might be attributed to the suppression of inflammatory mediators by alkaloids, flavonoids, glycosides, or steroids (Khan *et al.,* 2012).

Diabetes mellitus is a serious endocrine illness that affects around 10% of the global population (Burke *et al.,* 2003). Diabetes is among the primary causes of death in people and animals. Numerous studies have demonstrated that medicinal plants with hypoglycemic properties work through a variety of mechanisms, such as increasing insulin production, improving target cell insulin sensitivity, and promoting the regeneration of the β-cells in the pancreatic islets of Langerhans (Alam *et al.,* 2022). Flavonoids have also been shown to function as insulin secretagogues and repair injured beta cells in rats with diabetes induced by alloxan (Hussain *et al.,* 2022). It has been observed that polyhydroxylated alkaloids may be used therapeutically to treat type 2 diabetes because of their capacity to inhibit maltase-glucoamylase (Muhammad *et al.,* 2021). Crude fractions of *V.cotinifolium* contained quercetin, a flavonoid, according to phytochemical screening. In streptozotocin-induced diabetic rats, quercetin has been demonstrated to have antidiabetic effects by promoting insulin secretion through pancreatic islet regeneration (Latifi *et al.,* 2022). The hypoglycemic action of the crude fractions of *V.cotinifolium* may also be explained by these histological findings.

Conclusion

The findings of the current study using solvent extracts of leaves and stem of *V.cotinifolium* make it abundantly evident that this species may effectively to kill *Adies* larvae. The deadly concentration of the bioactive crude fraction that caused death was identified and extracted. Because it acts at a very low dose rate, it will be less expensive than synthetic insecticides. It is also readily available locally, readily biodegradable, and harmless. The study recommends identifying and including the active components in the composition of a commercial product intended to kill mosquitoes.

In current study the antidiabetic potential of the ethyl acetate fractions of stem and leaves of *V.cotinifolium* was studied and the fractions produced a significant anti-diabetic effect after 6 hours at the dose of 300mg/Kg body weight. These effects were well comparable with the standard drug glibenclamide used in the present study. The ethyl acetate fraction of stem was more effective anti-diabetic even in comparison to standard drug with 86.07 percent inhibition. The anti-diabetic activity shown by these crude fractions of stem and leaves is of considerable importance and justified its use as anti-diabetic in future with fewer side effects.

References

1. Alam, M., Uddin, G., Siddiqui, B. S., Sadat, A., Khan, A. A., & Rauf, A. (2014). Gas chromatography-mass spectrometry (GC-MS) analysis, antimicrobial, phytotoxic, insecticidal and leishmanicidal activities of fixed oil from Viburnum grandiflorum. *Afr. J. Pharm. Pharmacol*, *8*(46), 1173.
2. Alam, S., Sarker, M. M. R., Sultana, T. N., Chowdhury, M. N. R., Rashid, M. A., Chaity, N. I., ... & Mohamed, I. N. (2022). Antidiabetic phytochemicals from medicinal plants: prospective candidates for new drug discovery and development. *Frontiers in endocrinology*, *13*, 800714.

3. Burke, J. P., Williams, K., Narayan, K. V., Leibson, C., Haffner, S. M., & Stern, M. P. (2003). A population perspective on diabetes prevention: whom should we target for preventing weight gain?. *Diabetes care*, *26*(7), 1999-2004.
4. Campbell, F. L., Sullivan, W. N., & Smith, C. R. (1933). The relative toxicity of nicotine, anabasine, methyl anabasine, and lupinine for culicine mosquito larvae. *Journal of Economic entomology*, *26*(2), 500-509.
5. Chandra, S., Chatterjee, P., Dey, P., & Bhattacharya, S. (2012). Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein. *Asian Pacific Journal of Tropical Biomedicine*, *2*(1), S178-S180.
6. Dagli, N., Dagli, R., Mahmoud, R. S., & Baroudi, K. (2015). Essential oils, their therapeutic properties, and implication in dentistry: A review. *Journal of International Society of Preventive and Community Dentistry*, *5*(5), 335-340.
7. Fawole, O. A., Amoo, S. O., Ndhlala, A. R., Light, M. E., Finnie, J. F., & Van Staden, J. (2010). Anti-inflammatory, anticholinesterase, antioxidant and phytochemical properties of medicinal plants used for pain-related ailments in South Africa. *Journal of Ethnopharmacology*, *127*(2), 235-241.
8. Ghosh, A., Chowdhury, N., & Chandra, G. (2012). Plant extracts as potential mosquito larvicides. *Indian journal of medical research*, *135*(5), 581-598.
9. Grover, J. K., Yadav, S., & Vats, V. (2002). Medicinal plants of India with anti-diabetic potential. *Journal of ethnopharmacology*, *81*(1), 81-100.
10. Hajhashemi, V., Sajjadi, S. E., & Heshmati, M. (2009). Anti-inflammatory and analgesic properties of Heracleum persicum essential oil and hydroalcoholic extract in animal models. *Journal of ethnopharmacology*, *124*(3), 475-480.
11. Haldar, K. M., Ghosh, P., & Chandra, G. (2011). Evaluation of target specific larvicidal activity of the leaf extract of Typhonium trilobatum against Culex quinquefasciatus Say. *Asian Pacific Journal of Tropical Biomedicine*, *1*(2), S199-S203.
12. Hartzell, A. L. B. E. R. T., & Wilcoxon, F. (1941). A survey of plant products for insecticidal properties. 12:127–41.
13. Hasanpour, M., Iranshahy, M., & Iranshahi, M. (2020). The application of metabolomics in investigating anti-diabetic activity of medicinal plants. *Biomedicine & Pharmacotherapy*, *128*, 110-263.
14. Hussain, F., Hafeez, J., Khalifa, A. S., Naeem, M., Ali, T., & Eed, E. M. (2022). In vitro and in vivo study of inhibitory potentials of α-glucosidase and acetylcholinesterase and biochemical profiling of M. charantia in alloxan-induced diabetic rat models. *American journal of translational research*, *14*(6), 3824.
15. Kaneria, M., Baravalia, Y., Vaghasiya, Y., & Chanda, S. (2009). Determination of antibacterial and antioxidant potential of some medicinal plants from Saurashtra region, India. *Indian journal of pharmaceutical sciences*, *71*(4), 406.
16. Kaushik, D.; A. Kumar.; P. Kaushik.; A. C. Rana. (2012). Preliminary phytochemical screening of selected Medicinal Plants. *Journal of Chinese Integrative Medicine. 9*, 1056-1060.
17. Kishore, N., Mishra, B. B., Tiwari, V. K., & Tripathi, V. (2011). A review on natural products with mosquitosidal potentials. *Opportunity, challenge and scope of natural products in medicinal chemistry. Kerala: Research Signpost*, *11*, 335-65.
18. Latifi, E., Mohammadpour, A. A., Fathi Hafshejani, B., & Nourani, H. (2022). Ferula assa-foetida oleo gum resin ethanolic extract alleviated the pancreatic changes and antioxidant status in streptozotocin-induced diabetic rats: a biochemical, histopathological, and ultrastructural study. *Journal of Food Biochemistry*, *46*(8), e14191.
19. Matthew, S., A. K. Jain., M. James., C. Matthew and D. Bhowmik. 2013. Analgesic and anti-inflammatory activity of Kalanchoe pinnata (lam.) pers. *Journal of Medicinal Plants*, 1(2): 24-28.

20. Muhammad, I., Rahman, N., Nishan, U., & Shah, M. (2021). Antidiabetic activities of alkaloids isolated from medicinal plants. *Brazilian Journal of Pharmaceutical Sciences*, *57*, e19130.
21. Patidar, A., Birla, D., Patel, V., Chaturvedi, M., & Manocha, N. (2014). A Review on advantages of Natural Analgesics over Conventional Synthetic Analgesics. *International Journal of Pharmacy & Life Sciences*, *5*(5).
22. Saha, S. (2019). Phytochemical screening and comparative study of antioxidative properties of the fruits and leaves of Spondias mombin in Bangladesh. *J. Pharmacogn. Phytochem*, *8*, 379-383.
23. Selvam, C., & Jachak, S. M. (2004). A cyclooxygenase (COX) inhibitory biflavonoid from the seeds of Semecarpus anacardium. *Journal of ethnopharmacology*, *95*(2-3), 209-212.
24. Shaalan, E. A. S., Canyon, D., Younes, M. W. F., Abdel-Wahab, H., & Mansour, A. H. (2005). A review of botanical phytochemicals with mosquitocidal potential. *Environment international*, *31*(8), 1149-1166.
25. Su, S., Wang, T., Duan, J. A., Zhou, W., Hua, Y. Q., Tang, Y. P., ... & Qian, D. W. (2011). Anti-inflammatory and analgesic activity of different extracts of Commiphora myrrha. *Journal of ethnopharmacology*, *134*(2), 251-258.
26. Upadhaya, V. P., & Pandey, K. (1984). Ayurvedic approach to diabetes mellitus and its management by indigenous resources. Diabetes mellitus in developing countries. *New Delhi*, 54.
27. Wang, Z., Kim, J. R., Wang, M., Shu, S., & Ahn, Y. J. (2012). Larvicidal activity of Cnidium monnieri fruit coumarins and structurally related compounds against insecticide-susceptible and insecticide-resistant Culex pipiens pallens and Aedes aegypti. *Pest Management Science*, *68*(7), 1041-1047.
28. Warikoo, R., & Kumar, S. (2013). Impact of Argemone mexicana extracts on the cidal, morphological, and behavioral response of dengue vector, Aedes aegypti L.(Diptera: Culicidae). *Parasitology Research*, *112*, 3477-3484.
29. WHO Expert Committee. (1980). Diabetes Mellitus, 2nd Rep. Geneva. *World Health Organization (Technical Report Series). p*, *646*.
30. Zubairi, S. I., Sarmidi, M. R., & Aziz, R. A. (2004). A study into the effect of concentration process on the yield of rotenone from the extract of local plant species. In *Proceeding of 'Seminar Sebatian Smulajadi Malaysia Ke-20*.