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Analysis of bioactive composites and antiviral activity of *Iresine herbstii* extracts against Newcastle disease virus *in ovo*

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ABSTRACT

The study was implemented to actuate the qualitative and quantitative phyto constituents of *Iresine herbstii* extracts and its antiviral efficacy against avian ND virus. Among four tested solvents, the ethanolic extract of *Iresine herbstii* revealed the presence of highest quantity of all tested phytochemicals while petroleum ether extract showed the least. Folin-Ciocalteu method assessed the range of TPC extended from 81.01 ± 0.67 to 126.35 ± 0.45 μg GAE/mg. Acetonic extract showed the highest amount among all extracts and petroleum ether possessed the lower quantity. TFC ranged from 54.37 ± 0.45 to 88.12 ± 0.26 μg QE/mg followed by colorimetric method. From all extract ethanolic extract showed highest quantity and petroleum ether revealed the lower. HPLC analysis of ethanolic extract of *I. herbstii* confesses the presence six bioactive components by using the HP5-MS column. To check the antiviral potential of plants, different prepared treatments of plant extract and live virus were inoculated at 9 days old SPF embryonated chicken eggs. Results exposed that all plant extracts produce antiviral activity against NDV *in ovo* according to their potential and phytochemical profile. The highest survival rate was observed in the ethanolic extract at 400 μg/mL and acetonic extract at 300 μg/mL as it controls the NDV activity completely, evidence of absence of embryo death and HA titre. Dichloromethane and petroleum ether could not inhibit the virus completely. 600 μg/mL concentration was proved as toxic in all extracts except petroleum ether extract which showed a dose dependent pattern.

1. Introduction

Poultry industry was commenced as a cottage industry and play important role in many developing countries' economies throughout the world (Ismail, 2017). It is developed as the 2nd major industry and one of the affordable and considerable sources of protein as eggs and meat, about 40% meat requirement in Pakistan is bringing with it. But there is a long list of complications linked with the poultry industry (Hussain et al., 2015). Around all entire world, poultry has facing many socio-economic crisis due to viral poultry diseases with every passing year like Newcastle disease (ND) is one of them (Yune and Abdela, 2017). It was reported as a LISTED by Office International des Epizooties [OIE], due to its dreadful concerns (Boynukara et al., 2013).

ND viruses a negative sense, non-segmented, single- stranded encapsulated RNA virus from family *paramyxoviridae* (Ashraf et al., 2016). ND is prevalent to many regions of world. NDV instigated death rate in high susceptibility. According to International Cooperation in Animal Science (IICAB, 2016) 250 bird species of 27 orders are affected from this virus. It has become stern disease in poultry and killed unvaccinated rural poultry at rate of 70–80% per year. Concerning to pathological index NDV is categorized in three groups; lentogenic, mesogenic and velogenic. In Pakistan incidences of lentogenic, mesogenic and velogenic are 40%, 55% and 5% respectively (Waheed et al., 2013). Disease and death rate extent upto 100% in serious cases.

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Presently, many inactivated and live vaccines are present in market, but only few developed medicines proved effective (Xiao et al., 2013). Clinical signs can preclude by vaccination but replication and shedding of virus channot stop, that is main cause of infection (Chukwudi et al., 2012). To discover antiviral agents and new corresponding actions, that are safe, effective, produce less side effects and also overcomes resistance is the basic requirement of time (Nogon et al., 2011). An enormous number of different kinds of plants possessing an antiviral effect is present in rich and still fertile the nature. Each region has its own highly variable and special kind of plants which are not found in other region in world (Waziri, 2015). World Health Organization (WHO) estimated that about 75% of population fulfills their health care necessities by using herbs or plants based medicines (Ravindra et al., 2009). Owing to their availability, effective antimicrobial properties, low cost and enhancement in performance of commercial poultry birds, the medicinal plants are in use since many years in the world (Sajid et al., 2011).

Many Pakistan native plants promise immense properties for the discovery of new compounds with active medicinal functions in immune system. Prior investigation on plants showed different extracted phyto-components which are chief secondary metabolites like lignans, alkaloids, coumarins, triterpenoids, flavonoids, and chromones (Qasim et al., 2014). In accordance of worldwide trend, therefore, aimed at evaluating the antiviral efficacy of *Iresine herbstii* (chicken gizzard plant) against NDV. *Iresine herstii* is the member of *Amaranthaceae* family. Commonly it is called as chicken gizzard, blood leaf, herbst's blood leaf and beef steak. It is intrinsic to South America also found in tropical areas of Asia and India. It has multiple applications in folk medications (Chaudhuri and Sevanan, 2012). Research revealed that this plant used decoction relaxant, fever and kidney disorders also retain anti-allergic, anticancer, anti-inflammatory, antipyretic, little antioxidant, apoptotic and cytotoxic abilities (Schmidt et al., 2009). Reason for selecting plant is that plants are easily affordable and accessible and can subscribe to new bioactive constituents that are safe and effective. This make them offered for farmers easily.

The objective of study is to preliminary phytochemical profiling of shoot part of four extracts of *Iresine herbstii* qualitatively, total phenolic and total flavonoid contents (quanitative), detect bioactive composite by HPLC and antiviral activity of its ethanolic, acetonic, dichloromethane and petroleum ether extracts against NDV in embryonated chicken eggs *in ovo*.

2. Materials & methods

2.1. Plant collection and identification

Clean, hygienic and disease free shoot part *Iresine herbstii* was collected from Botanical garden of Government College University Faisalabad (GCUF). According to standard descriptions and keys plant was identified in field (Dalziel, 1937). Botanical identity was authenticating by Department of Botany, GCUF. After collection the leaves and stem of plant were dried and then pulverized them in the grinder.

2.2. Maceration

Following the method designated by Dipankar and Murugan (2012) extraction of solvent was carried out. Plant was macerate at room temperature for 3 days by assorted separately in four different solvents as Acetone, Dichloromethane, Ethanol and Petroleum ether 500 g/1 L for each concentration. Then filter the macerated solution by What Mann's paper. Now the filtrate was concentrated in rotary evaporator to viscous the extract until all the ethanol (or other solvents) was cleared. Stock solutions were prepared by dissolving the crude extracts in Dimethyl sulphoxide (DMSO) solution. Then different experimental concentrations of plant extracts were prepared to estimate its antiviral efficacy. The crude extracts can be stored at 4 °C in airtight bags for future use.

2.3. Sterility test

Poured 0.1 mL of all extract on a nutrient agar plate and left for 2 min. Incubated overnight at 37 °C to observe the antibacterial activity (Ashraf et al., 2017).

2.4. Phytochemical screening

Phytochemical profiling of different extract of *I. herbstii* was executed by following standard assays (Ayoola et al., 2008; Joshi et al., 2013).

2.5. Total phenolic quantitation

Method adopted by Chlopicka et al. (2012) was followed for determination of total phenolic contents of all extracts of *I. herbstii*. 1500 μL of 10 fold diluted Folin-Ciocalteu reagent mixed with 300 μL of extract. 1200 μL of Na_2CO_3 was added after 5 min and kept the mixture in dark room for 2 h. Gallic acid was used as standard for calibration curve. Then observe the absorbance at 765 nm by spectrophotometer and results were expressed as Gallic acid equivalent (μg GAE/mg).

2.6. Total flavonoids quantitation

Procedure described by Stankovic (2011) was adopted to estimate TFC of *I. herbstii* extracts. One mL extract was mixed with 1 mL of 2% $AlCl_3$. Incubate it for 10 min at 37 °C. Quercetin was used as a standard for calibration. Checked the absorbance was determined at 415 nm by spectrophotometer (Thermo, Waltham, MA, USA). Results were expressed as Quercetin equivalents (μg QE/mg).

2.7. High-performance liquid chromatography (HPLC)

2.7.1. Sample preparation

Reverse phase chromatographic analyses were carried out under gradient conditions using C_{18} column (4.6 mm × 250 mm) packed with 5 μm diameter particles. The extract was prepared in HPLC grade solvent. Then, the sample was sonicated using ultra sonicator for 10 min. The extract was filtered and injected into the HPLC column using mobile phase of 30:70 (acetonitrile:0.1% phosphoric acid).

2.8. In ovo antiviral assay

The Lasota strain (least virulent/avirulent strain of NDV) was purchased from Qadri market, Gol bazar, Faisalabad, Pakistan. Specific pathogen free embryonated chicken eggs (ECE) were incubated for 9 days in incubator at 37 °C. Median Embryo infectious dose (EID_{50}) of the virus was determined as documented by (Young et al., 2002). From this, 100EID_{50}/0.1 mL of the virus stock was prepare for the trial. The index calculated by Reed and Muench formula.

2.8.1. Inoculation of eggs

The SPF embryonated eggs for every extract (ethanolic, acetonic, dichloromethane and petroleum ether) were divided into seven groups (five eggs in each group) and labeled according to

Table 1
Grouping and treatment allocation for different concentration of four extracts of *Iresine herbstii in ovo.*

Group (G) n = 7	Treatment
G1	Ethanol/Acetone/DCM/PE extract of *Iresine herbstii* 300 μg/mL + 0.2 mL 4HA Virus
G2	Ethanol/Acetone/DCM/PE ether extract of *Iresine herbstii* 400 μg/mL + 0.2 mL 4HA Virus
G3	Ethanol/Acetone/DCM/PE ether extract of *Iresine herbstii* 500 μg/mL + 0.2 mL 4HA Virus
G4	Ethanol/Acetone/DCM/PE ether extract of *Iresine herbstii* 600 μg/mL + 0.2 mL 4HA Virus
G5	Virus control
G6	Extract control
G7	Untreated embryonated chicken egg

DCM = Dichloromethane; PE = Petroleum Ether.

concentration of extract used (Table 1). Pinch a hole in shell just above the air sac to let vertical inoculation of 0.1 mL of the inoculum. In all different extract groups, Group 1 (G1) to G4 was inoculated with 0.2 mL of virus/extract mixtures at final concentration with 300 μg/mL, 400 μg/mL, 500 μg/mL, 600 μg/mL for *I. herbstii* ethanolic extract. G5 inoculated with only 0.2 mL of virus and took as positive control. G6 has eggs inoculated with extract only (no virus). In G7 all eggs were uninoculated (negative control). Same concentrations were taken for other extracts. Inoculated sites were closed with paraffin then incubated at 37 °C for 96 h. Tested Eggs were perceived daily for death of embryo till 72 h post inoculation. After 96 h, cooled the eggs and embryos were perceived for survival & growth. Collect allantoic fluid from treated eggs haemagglutination test to detect NDV.

2.8.2. Hemagglutination test

To quantify the sum of virus, HA titre test was used by using two fold dilutions. In sterile plastic bottom allantoic fluid was put by harvesting it from surviving embryos eggs. From harvested allontoic 50 μL was serially diluted by 50 μL of normal saline. In 96 well V bottom designed microtiter plate. Then, to each well 50 μL of RBCs 1% (freshly collected from chicken) was added and mixed gently. Allow them to stand for 25 min at room temperature. After sometime virus titre was noted as reciprocal of highest dilution that triggered agglutination of chicken RBCs (Khaldoun, 2016).

2.9. Statistics analysis

The results were evaluated using Minitab statistics- 17. The differences were analyzed by analysis of variance (ANOVA) and significance was reported at $P \leq 0.05$. The square root values of HA titre were converted to standardize the data before put in it into regression analysis.

3. Results

3.1. Qualitative phytochemical screening

The preliminary qualitative phytochemical screening for all sequential of *Iresine herbstii* extracts reveals the incidence of alkaloids, anthraquinones, glycosides, flavonoids, saponins, phenols, terpenoids, sugar bearing compound, protein, thiols and inferences are shown in Table 2.

3.2. Total phenolic quantitation

The total phenolic contents intended against Gallic acid Equivalent (GAE) are shown in Table 3. The phenol contents range from

Table 2
Qualitative phytochemical analysis of ethanolic, acetonic, dichloromethane & petroleum ether extracts of *Iresine herbstii.*

Phytochemicals	Test Type	Inferences			
		Ethanol	Acetone	DCM	PE
Alkaloids	i.Dragendroff's test	+	+	+	+
	ii.Mayer's test	+	+	+	+
	iii.Wagner's test	+	+	+	+
Flavonoids	NaOH Test	+	+	+	+
Glycosides	Keller–Kiliani's Test	+	+	+	+
Phenols	Ferric chloride Test	+	+	+	+
Protein	Ninhydrin Test	+	+	+	–
Reducing sugar	Fehling Test	+	+	+	–
Tannins	Ferric chloride Test	+	–	–	–
Terpenoids	Salkowski's Test	+	+	–	–
Thiol		+	+	+	–
Saponins	Froth Test	+	+	+	–
Anthraquinones	Borntrager's Test	+	+	+	–

(+) = present, (–) = absent.

Table 3
Total phenolic contents and total flavonoid contents of *I. herbstii* shoot part of different extracts.

Extracts	Total phenolic contents (μg GAE/mg)	Total flavonoid contents (μg QE/mg)
Ethanolic	98.00 ± 0.05	88.12 ± 0.26
Acetonic	126.35 ± 0.45	72.01 ± 1.00
Dichloromethane	89.05 ± 0.15	68.19 ± 2.55
Petroleum ether	81.01 ± 0.67	54.37 ± 0.45

81.01 ± 0.67 μg GAE/mg to 126.35 ± 0.45 μg GAE/mg. Acetonic extract showed the highest amount among all four extracts and petroleum ether possessed the lower quantity of TPC. Phenols are considered as central compound due to their reducing/scavening capacity. The interest on phenols and polyphenolic compounds such as flavonoids which possess antioxidant ability is increases in food industry because they slow down the oxidative degeneration of lipids thus improving the quality and nutritive value of food. Therefore, it is justifiable to determine phenolic content in plant extract.

3.3. Total flavonoids quantitation

Flavonoids are commonly found in natural products and have great importance because they help human body to fight against diseases. Among different extracts of shoot part of *I. herbstii* flavonoids are raged from 88.12 ± 0.26 μg QE/mg to 54.37 ± 0.45 μg QE/mg. Ethanolic extract showed highest quantity and petroleum ether revealed the lower shown in Table 3.

3.4. HPLC analysis

HPLC is an interesting technique to identify the volatile matter, long chain, branched chain hydrocarbons, alcohols acids, esters, etc. with respect to Peak area, retention time and molecular formula in plant compound library (as shown in Fig. 1 and Table 4) which are active bioconstituents in drugs, food, pharmaceutical and cosmetic industry. It is evident from the Table 4 that the ethanolic extract has a complex chemical composition. Six bioactive molecules are identified among these Dimethyl Sulfaxide, 1H-Imidazale, Silicic acid, diethyl bis(trimethylsilyl) ester, Cycloheptasiloxane tetradecamethyl-, cyclononasiloxane octadecamethyl-, cyclodecasiloxane eicosamethy- are present. Some of the HPLC peaks were not identified because of lack of authentic library data to corresponding compounds. A general observation is that the quantity of the aromatic compounds is

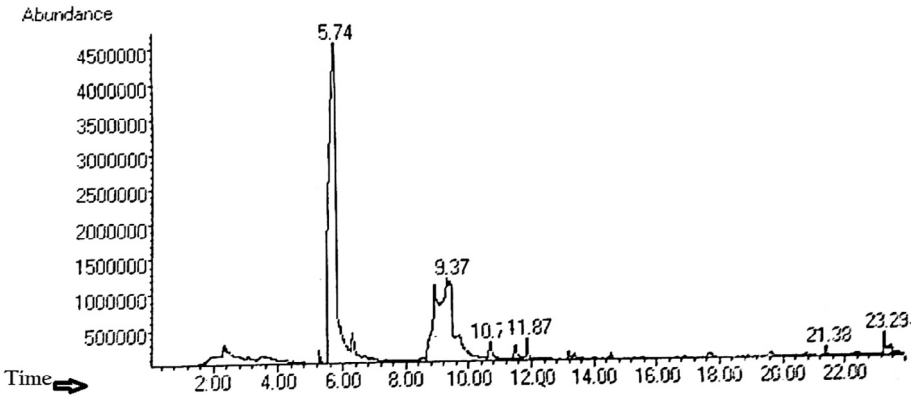

Fig. 1. HPLC Chromatogram of shoot part of *I. herbstii* ethanolic extract.

Table 4
List of probable phytochemical constituents identified by HPLC spectra.

Peak No.	Retention Time	Compound Found	Area %	Molecular formula	Molecular weight
1	5.737	Dimethyl Sulfaxide	58.16	C_2H_6OS	78.13 g/mol
2	9.368	1H-Imidazale	35.67	$C_3H_4N_2$	68.079 g/mol
3	10.704	Silicic acid, diethyl bis(trimethylsilyl) ester	1.89	$C_{10}H_{28}O_4Si_3$	296.585 g/mol
4	11.867	Cycloheptasiloxane tetradecamethyl-	1.99	$C_{14}H_{42}O_7Si_7$	519.078 g/mol
5	21.383	cyclononasiloxane octadecamethyl-	0.47	$C_{18}H_{54}O_9Si_9$	667.386 g/mol
6	23.292	cyclodecasiloxane eicosamethy-	1.79	$C_{20}H_{60}O_{10}Si_{10}$	741.54 g/mol

Table 5
Median percent Embryo Infectious Dose (EID_{50}/mL).

EID_{50}/mL Dilutions	No. of eggs	No. of eggs alive	No. of eggs dead	Percentage Mortality
10^{-1}	5	0	5	100%
10^{-2}	5	2	3	60%
10^{-3}	5	3	2	40%
10^{-4}	5	5	5	60%
10^{-5}	5	5	0	0%
10^{-6}	5	5	0	0%
10^{-7}	5	5	0	0%
10^{-8}	5	5	0	0%
10^{-9}	5	5	0	0%
10^{-10}	5	5	0	0%
Virus control	5	0	5	100%
PBS control	5	5	0	0%

more than that of aliphatic compounds and these are mainly phenolic and flavonoids (see Table 5).

3.5. Median percent embryo infectious dose

The concentration of NDV in a suspension is expressed as an Infectivity titre. The Infectivity titre is established by performing a titration. Table 3 shows the results of titration values by Reed and Muench formula are as follows:

$$\textbf{Dilution} = \frac{\%\ \text{Mortality at dilution just above 50\%} - 50\%}{\%\ \text{Mortality at dilution just above 50\%} - \%\ \text{Mortality at dilution just below 50\%}}$$

Apply the index calculated using this formula to the dilution that produced the infection rate immediately above 50 percent = $10^{-3.5}$. This dilution of the virus suspension contained one EID_{50} unit of virus in 0.2 mL. 1 mL of the virus suspension will contain ten times the reciprocal of the calculated dilution.

Therefore infectivity Titre of virus suspension in EID_{50}/mL = $10 \times 10^{-3.5} = 10^{-4.5}$ EID_{50}/mL.

3.6. In ovo antiviral activity of different extracts of Iresine herbstii

In this study, all chicken embryos dead within 48 h post inoculation with NDV, it was a strong warning that the virus strain is virulent to eggs. However, addition of any four tested extracts of *Iresine herbstii* significantly prolonged the time of survival of embryos in dose dependent manner. In ethanolic extract treated group concentration of 400 μg/mL had complete inhibition of NDV replication with 0% mortality (see Table 6). Heamagglutination showed 0 HA titre it means that this concentration controlled virus from beginning. 300 μg/mL and 500 μg/mL had 20% mean mortalities at 48 h and has no mortality till 72 h. 300 μg/mL has 4 HA titre, it control the virus replication earlier than 500 μg/mL which have Heamagglutionation at 8. Highest concentration (600 μg/mL) cause 40% mortality after 72 h post inoculation with HA titre of 32 (Table 7). It may suggest that all the concentrations have ability to inhibit virus multiplication.

In acetonic extract treated group eggs, 300 μg/mL inoculated group showed 0% mortality throughout three days (72 h) post inoculation showing HA titres of 0. 400 μg/mL and 500 μg/mL inoculated group have 20% mortality indicating that both have same capability to control the virus. High death rate was observed in 600 μg/mL treated group and showing the HA titre is 128. It can

Table 6
Embryo deaths following inoculation of embryonated chicken eggs with Newcastle disease virus (NDV) and different concentrations of different extracts of *I. herbstii* shoot part.

Treatment	Concentration (μg/mL)	Mortality with different time intervals			% Mortality
		24 h	48 h	72 h	
Ethanolic Extract	300	0	1	0	20%
	400	0	0	0	0%
	500	0	1	0	20%
	600	0	1	1	40%
Acetonic Extract	300	0	0	0	0%
	400	0	1	0	20%
	500	0	0	1	20%
	600	0	1	2	60%
DCM Extract	300	0	1	1	40%
	400	0	0	1	20%
	500	0	1	1	40%
	600	1	1	1	60%
PE Extract	300	1	1	1	60%
	400	1	2	0	60%
	500	0	1	1	40%
	600	1	1	0	40%
VC	_	2	3	_	100%
EC	_	0% mortality.			0%
Uninoculated Group	_	0% mortality.			0%

DCM = Dichloromethane; PE = Petroleum Ether; VC = Virus control; EC = Extract Control

Table 7
Mean hemagglutination (HA) titres in embryonated chicken eggs inoculation with NDV and different concentrations of different extracts of *Iresine herbstii*.

Treatment	Concentration (μg/mL)	HA titre
Ethanolic Extract	300	4
	400	0
	500	8
	600	32
Acetonic Extract	300	0
	400	4
	500	8
	600	128
Dichloromethane Extract	300	32
	400	8
	500	128
	600	512
Petroleum Ether Extract	300	1024
	400	512
	500	512
	600	128
Virus Control	_	2048
Extract Control	_	0
Uninoculated Eggs	_	0

be interpreted that this dose has a lethal effect itself on embryo survival (Tables 6 and 7). Findings are in line with results of Ashraf et al. (2017) who documented that low concentrations 300 μg/mL of methanolic and 400 μg/mL ethanolic extract of *Glycyrrhiza glabra* produce zero % mortality. High mortality was shown by 600 μg/mL of different extract at 24 h, 48 h and 72 h time intervals.

The three days record of dichloromethane extract of *I. herbstii* inoculated group indicated that there is no complete restriction of antiviral potential was noticed using different concentrations of plant extract. There is no mortality at 24 h in first three concentrations. After 48 h there is 1 embryo died in both 300 μg/mL and 500 μg/mL. Total mortality is 40% till 72 h in both groups. HA titre showed different titre values regarding to their aptitude to control the viral effects. 600 μg/mL has 60% mortality 72 h post inoculation. From the results it may be concluded that dichloromethane extract of this plant has less ability to control the virus propagation.

Petroleum ether extract of *I. herbstii* displayed the dose dependent response by level of virus control. In this extract 300 μg/mL and 400 μg/mL have shown 60% mortality (Table 6). In. HA titre 1024 of 300 μg/mL indicates that this concentration of PE did not control virus replication while 400 μg/mL group showed HA titre 512. 500 μg/mL and 600 μg/L has less mortality rates than first two concentrations i.e., 40%. These outcomes are in line with findings of Al- Hadid (2016), who conducted similar work and stated that leaves extract of *Ceratonia siliqua* at any of plant concentrations did not inhibit NDV activity completely. High concentration 500 μg/mL showed 20%, 250 μg/mL showed 40% and low concentration of *Ceratonia siliqua* caused 80% mortality.

4. Discussion

The use of medicinal plants in dealing of ailments has provoked renewed attention in recent years, herbal preparations are progressively being used in human and animals healthcare schemes. Use of the extracts from *Iresine herbstii* for cure of different maladies has been documented (Chudhuri and Sevanan, 2012). It can be predicted that antiviral potential of plant could be attributed the presences of secondary metabolites compounds (Chadare et al., 2009).

The mechanism conduct by the crude extracts of *I. herbstii* to control *in ovo* NDV replication is not known yet. This exposes the inhibitory rather than virucidal potential of the extract on this virus at these doses. However, many traditional medicinal plants that are used to cure viral diseases have been exposed to contain different types of substances. Notable examples of these metabolites comprise; alkaloids, coumarins, terpenes, flavonoids, anthraquinones and naphthoquinones (Sulaiman et al., 2011). These compounds exert their effects by killing the virus and interfering with viral replication (Jassim and Naji, 2003).

Huge number of small phytochemicals such as alkaloids, flavonoids, phenols, sugar bearing molecules and terpenes were found to containing anti-herpetic, anti-inflammatory, anti-viral, anti allergesic, anti-cancer, anti-bacterial, anti-malarial and anti-fungal agents (Viol, 2013). Plant having glycosides and carbohydrates which are considered possessing a helpful immune mechanism by enhancing body power and dietary additives (Yadav et al., 2014). Proteins added structural and functional improvements in plants as well as animal cells and have nutritional values (Tijjani et al., 2013). Glycosides were also predicted to be anti diarrheal effects because of prevention of autocoids and prostaglandin (Tiwari et al., 2011).

Plants have natural antioxidants in the form of phenolic and polyphenolic compounds (flavonoids), which presence provide essential plant biochemistry and physiological effects such as antioxidant, anti-inflammatory, cytotoxic efficacy and anti-allergic effects (Kumar and Pandey, 2013), antidiabetic, anti-carcinogenic activity, enzyme inhibitor and precursor of toxic substance (Prabhu et al., 2012).

Acetonic extract and ethanolic extract at 300 μg/mL and 400 μg/mL exhibited the complete inhibition of NDV without prompting mortality of any chicken embryo. The response of viral infected embryo is different depending on extract type and concentration supplied. Some extracts of plant expressed dose dependent relationship with virus while other cause toxicity for embryos. The positive responses provided by extract are believed to be as an effect of phytochemicals present in that extract or combination with other compounds. The belief that medicinal plants don't have side or toxic effects because they are natural so it is clear that toxicological effects may related with dose concentration. Dose dependent toxicological data should be provided while developing new drugs in future.

5. Conclusion

The compounds acknowledged by qualitative and quantitative investigations with HPLC have many uses in medical field. Every compound recognized has their unique characters to treat different ailments. Further investigations expected to uncover its significance in explicit field to treat the infections legitimately. The antiviral assay of *Iresine herbstii* reveals the significant antiviral potential of ethanolic and acetonic extracts against experimental Newcastle disease infection in local chickens. Administrations of this extract might be a healthier approach in lessening the effects of Newcastle disease virus infection. The positive responses provided by extract are believed to be as an effect of phytochemicals present in that extract or combination with other compounds. Therefore, *Iresine herbstii* is recommended as a plant of antiviral importance.

Declaration of Competing Interest

There were no competing interests among authors.

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