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Characterization and bioactivities of *M. arvensis, V. officinalis and P. glabrum*: In-silico modeling of *V. officinalis* as a potential drug source

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ABSTRACT

In current study the pharmaceutically active herbs was used against coccidiosis, caused by a protozoan: *Eimeria*, lead to $ 3 billion loss annually. The aqueous and methanolic extracts of whole plants were applied *in-vitro* to assess sporulation inhibition (spi) assay and calculated the inhibitory concentration (IC_{50}). For *in-vivo* study 9 groups of 14 day old broiler chicks were infected with *Eimeria tenella* and three groups were treated different concentrations of methanolic extracts of *Verbena officinalis* and *Polygonum glabrum* post infection. The mean weight gain, oocyst count, diarrhea, biochemical tests, hematology, and histopathology of all groups were analyzed. The herbs were characterized by antioxidant assay, phyto-chemical screening, Fourier transmission and infrared (FT-IR), Ultra Violet-visible (UV–Vis) spectroscopy and Gas chromatography and mass spectroscopy (GC–MS). The GC–MS identified phyto-compounds of *V. officinalis* were docked with S-Adenosyl methionine (SAM) synthetase. The *in-vitro* study revealed that *V. officinalis* and *P. glabrum* have minimum IC_{50} of 0.14 and 12 mg/ml respectively. The *in-vivo* experiment showed that *V. officinalis* had significantly high anticoccidial potential with significant hematological profile like drug treated controls. The histology of treated chicks also showed recovery in the studied tissues. The antioxidant assay showed that *V. officinalis* have 4.19U/mg Superoxide dismutase (SOD) and 33.96 μM/mg Glutathione (GSH) quantities. The chemical characterization confirmed the presence of large number of organic compounds, however Flavonoids found only in *V. officinalis*, which suggests the anticoccidial potential of *V. officinalis* because flavonoids as antagonist of thiamine (Prinzo, 1999), because it promotes the carbohydrate synthesis required. Strychane, 1-acetyl-20a-hydroxy-16-methylene has best binding of with target protein with lowest binding score (-6.4 Kcal/mol), suggests its anticoccidial potential in poultry.

Abbreviations: SOD, Superoxide dismutase; GSH, Glutathione; spi, sporulation inhibition; sp, sporulation; MWG, Mean weight gain; MIW, Mean initial weight; MFW, Mean final weight; WDB, weight of dead birds; opg, oocysts per gram; FCR, feed consumption ratio; SQX, sulfaquinoxaline; K2Cr2O7, Potassium dichromate; AST, Aspartate aminnotransferase; ALT, Alanine transaminase; SAM, S-Adenosyl methionine; IC, inhibitory concentration; wbc, white blood cell; rbc, red blood cell; Hb, hemoglobin; FT-IR, Fourier transmission and Infrared spectroscopy; UV-Vis, Ultra voilet Visible; GC-MS, Gas chromatography and mass spectroscopy.

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1. Introduction

Coccidiosis is a protozoan parasitic infection caused by seven species of Apicomplexa belonged to genus *Eimeria*, and family Eimeridae (Bahadoran et al., 2014), found across the world (Zaman et al., 2012). Coccidiosis is economically important disease in poultry sector, causes US $ 3 billion loss annually (Pawestri et al., 2020; Quiroz and Dantan, 2015) and almost US $ 0.8 billion is spent on prophylactic in-feed drugs (Allen and Fetterer, 2002). In Pakistan, avian coccidiosis was not studied enough but institutional-based surveys revealed 32.79% prevalence on average in fecal and litter observation, the molecular study confirmed the presence of *E. tenella, E. acervulina*, *E. necatrix, E maxima*, *E brunette, E. mitis* and *E. praecox* (Ayaz et al., 2003; Khan et al., 2006; Mustafa and Ali, 2005; Abbas et al., 2015; Sultana et al., 2009; Bachaya et al., 2012; Bachaya et al., 2015; Awais et al., 2012; Shamim et al., 2015; Amin et al., 2014; Rashid et al., 2019; Ali et al., 2014; Jamil et al., 2016; Akram et al., 2018; Naveed and Faryal, 2019; Yousaf et al., 2018; Sohail et al., 2019; Khan, 2019; Ullah et al., 2020a, 2020b). *Eimeria* spp are specific to different loci in the gastrointestinal tract of the avian host (Lee and Lee, 2007; Bachaya et al., 2015). About 1700 species have been reported in various organisms, but only nine are responsible for avian coccidiosis (Bachaya et al., 2015). Among these *E. tenella*, *E. maxima*, *E. brunette*, and *E. necatrix* are highly pathogenic, especially *E. tenella* which is specific to the ceca of the intestine (Nematollahi et al., 2009; Jadhav et al., 2011). The oocysts' contaminated water and feed are the main cause of coccidiosis causing issues like ruffled feathers, bloody diarrhea, dysentery, poor growth, decreased egg production, malabsorption, and loss of appetite (Gharekhani et al., 2014; Usman and Diarra, 2008). It alters the level of various macromolecules inside the host body, like lowering protein and carbohydrates, which ultimately results in weight loss (Sharma et al., 2015). Poultry farming has progressed to large-scale industries over time with the increasing population in Pakistan. However, the industry is continuously under the threat of parasitic infections and coccidiosis is one of them that cause chronic damage to the digestive and excretory tract of chicks (Nahed et al., 2022; Abbas et al., 2010), Adding to it, some other factors like low-quality feed, poor ventilation, farm density and compromised immunity make the host more vulnerable to coccidiosis (Hafez et al., 2020). The clinical infection result in mass mortality; or in the case of birds' survival or subclinical infection, the productivity of farm is reduced to some extent (Iqbal et al., 2014). To encounter avian coccidiosis, several strategies have been adopted but failed due to one reason or another (Peek and Landman, 2011). More than 230 tons of coccidiostats are used in the United Kingdom each year (Shirley et al., 2007), with a wide range of ionophores and synthetic drugs. In addition to the phenomenon of drug resistance, the second most common factor that limits the use of synthetic coccidiostats is the occurrence of residues or secondary metabolites in poultry products, possibly the reason for toxicity in secondary consumers as mentioned earlier (Clarke et al., 2014). The direct intake of these coccidiostats lead to serious complications like a cardiac failure or muscle problem but the residual form in meat or egg may have long-term effect on immune and reproductive systems (Roila et al., 2019). Several expensive synthetic coccidiostats have been produced with good results initially in terms of weight gain and FCR (Feed Conversion Ratio) but later on, these compounds results in serious problems like nicarbazin decreases egg-laying ability in hens and infertility (Sherwood et al., 1956; Chelazzi et al., 2000), amprolium leads to cerebrocortical necrosis in calves (Alexander, 2009), whereas the drugs like clopidol, diclazuril, maduramycin and salinomycin have negative impact on visceral organ especially intestine, it weakens the intestinal wall, slowing down the reabsorption of nutrients and eventually results in low weight gain (Bahadoran et al., 2014; Hassanpour et al., 2010). Along with expensiveness, the inadvisable use of these coccidiostats creates drug resistance in the *Eimeria* spp., which is unaffordable in small-scale poultry farming (Nahed et al., 2022).

To avoid the use of such products, scientists worked on natural products to control coccidiosis, some of the plants or plant derivatives used in this scenario are artemisinin, *Curcuma longa*, *Saccharum officinarum* (Khalafalla et al., 2011; Awais et al., 2011). Besides these, some probiotics derived from fungi and bacteria have also been used in past (Elmusharaf et al., 2006; Jamil et al., 2017). Unlike synthetic drugs, plant extracts usually don't have adverse effects on the consumer and their adequate usage is beneficial, with easy availability and low cost (Karimi et al., 2015).

Verbena officinalis L. (Verbenaceae) is a cosmopolitan herb commonly known as vervain, enriched with sterols, triterpenic acids, caffeoyl derivatives, flavonoids, phenolic acid, verbascoide and iriods (Akerreta et al., 2007; Guarrera et al., 2005; Casanova et al., 2008). For long, *V. officinalis* is used for medicinal purposes, like curing rheumatic pain and thyroid problems, stress and insomnia, as an analgesic agent, antifungal and antibacterial agent and anticancerous, as culicide respectively (Akerreta et al., 2007; Guarrera et al., 2005; Williams et al., 2006; Agelet and Valles, 2011; Calvo, 2006; Hernandez et al., 2000; Ahmed et al., 2012; Pavela, 2009; Encalada et al., 2015). *P. glabrum* (Dense flower knotweed) is a perennial herb found on the river banks in South Asian countries (Raja and Ramya, 2017; Malik et al., 2018). The presence of zinc, copper, iron, and vitamins in its phytochemical composition enhances its medicinal value (Bhati and Jain, 2016). The b-hydroxyfriedalanol extracted from *P. glabrum* was found active against HIV (Said et al., 2015). It has shown antileishmanial (Rahman et al., 2015), antifungal, antibacterial activity (Palani et al., 2014), and antioxidant potential (Babitha et al., 2012). *Mentha arvensis* (Lamiaceae) is a cosmopolitan herb (Thawker et al., 2016), exhibits antibacterial property (Naseem et al., 2022), antioxidant (Biswas et al., 2014), anticandid potential (Santos et al., 2012) and is also used against digestive infection in human (Londonkar and Poddar, 2009). As a methyl donor, SAM methylates the DNA and switched off the genes. So, SAM controls genes expression (Reytor et al., 2009). SAM play important role in cell metabolism, therefore blocking the active site of SAM synthetase can inhibit the SAM synthesis which in turn will stop the metabolic activities of cell and eventually the organism will not survive (Lu and Markham, 2002). It is conserved throughout the organisms (Garrido et al., 2011). Previously, the SAM synthase of *Eimeria* sp. was used as drug target to control the coccidiosis in poultry (Maheswari and Revathi, 2017). In the current study the anti-coccidiosis potential of aqueous and methanolic extracts of *V. officinalis*, *M. arvensis*, and *P. glabrum* have been tested against sporulating oocysts (*In-vitro*), and the methanolic extracts of *V. officinalis* and *P. glabrum* was applied for an *in-vivo* experiment in chicks, The *in-silico* modeling of GC–MS identified phyto-constituents of methanolic extracts of *V. officinalis* were done.

2. Materials and methods

2.1. Bioethical approval

The study was approved by bioethical committee of Quaid-i-Azam University and assigned a protocol number BEC-FBS-QAU2022-418.

2.2. Plants Sampling, extraction and phytochemical screening

The aerial parts of selected herbs (Fig: Si-1) were collected from district Mardan, Pakistan (34.3410°N, 72.2897°E). The flora were

washed, shade dried (27–37 °C), grounded, and passed through 60 hole/cm mesh to obtain fine powder. The crude methanolic and aqueous extracts were prepared from respective plant powder using soxhlet extraction apparatus (Shanghai^R Heqi, China) by taking 30 gm powder in filter paper and 300 ml solvent at 65 °C for 6 h. The extracts were concentrated by evaporating solvents using a rotary evaporator (RE-5299). The crude extracts were stored in the refrigerator at 4 °C. The stock solutions of each extract were prepared by dissolving 1gm of crude extract in 100 ml of distal water, which is further diluted into 5, 10, and 15 mg/ml using standard dilution formula i.e. $V_1 = C_2 \times V_2/C_1$ (Shah et al., 2017). Whereas V_1: Volume to be removed, V_2: Required volume, C_1: Stock solution concentration and C_2: Required Concentration. The percent extract yield of each experimented herb was calculated in grams by using the standard formula (Nwonuma et al., 2019).

$$PercentYield = \frac{ActualdryYield(g)}{Plant\ Powder\ used\ (g)} \times 100$$

2.3. Quantitative analysis, FT-IR and UV visible spectroscopy

The presence of Phenols, Flavonoids, Steroids, Glycosides, Alkaloids, Tannins, Plobatannins, Anthocynins, Leucoanthocynins, Couramins and Anthraquinones in the herbal extracts was evaluated by different phytochemical tests mentioned in table: Si-1 (Roghini and Vijayalakshmi, 2018).The functional groups of the organic compounds were characterized by FT-IR spectroscopy (Bruker Platinum ATR) in the range of 4000 to 500 cm^{-1} in the selected plants extract (Ullah et al., 2020a, 2020b). The proximate analysis of the respective plants was conducted using a spectrophotometer (Cecil CE-7400) with 2 mm width slit using 10 mm cell under UV–Vis light from 200 to 800 nm wavelength to calculate the band gap with the help of absorption spectra. The samples were diluted with same solvent (Jain et al., 2016).

2.4. GC–MS analysis of V. officinalis

The phytochemical investigation of methanolic crude extract of *V. officinalis* was carried out with Thermo MS DSQ (version: 2.0.7) equipment. The conditions followed were according to standard protocol, TR 5-MS capillary column with 30Mts, ID: 0.25 mm, Film thickness: 0.25 mm. Flow rate of mobile phase (carrier gas: He) was set at 1.0 ml/min dimensions. In GC part temperature programmed was 40 °C, gradually raised to 250 °C at 5 °C/min. The sample injection volume was 1 ml and run at the range of 50–650 *m/z*. The mass spectrum was analyzed using the Main EI-MS Library (mainlib) data base with 242,464 reference spectra and Replicate spectra Library (replib) with 33,782 reference spectra (Kanthal et al., 2014).

2.5. In-vitro bioassay

2.5.1. Antioxidant potential

The antioxidant potential of these extracts was analyzed by estimating the level SOD and GSH along with total protein concentration. The SOD level was determined by standard protocol (Mathew et al., 2011) using nitroblue tetrazolium reagent at 560 nm with the help of spectroscopy. The GSH was determined using the method defined by Jollow and coworkers (Jollow et al., 1974) with the wavelength of 412 nm. The total protein content of the plant extracts was estimated by the standard Bradford assay (λ = 595 nm).

2.5.2. Sporulation inhibition assay

The *in-vitro*, anticoccidial potential of the *P. glabrum*, *V. officinalis,* and *M. arvensis* was measured by inhibition of oocyst sporulation. The experiment was carried out in triplicate in Petri dishes, provided with 3 ml of concentrations (40, 20, 10, 5, and 2.5 mg/mL), inoculated with 1500 unsporulated oocysts, and incubated at room temperature. The Petri dishes were covered with perforated aluminum foil to maintain oxygen supply. In parallel, $K_2Cr_2O_7$ (Potassium dichromate) treated group was kept as negative control and sulfaquinoxaline (SQX) treated group as a positive control, which inhibits sporulation. The percent sporulated and unsporulated oocysts were counted with a neubauer chamber after 48 and 72 h of incubation under a stereo microscope (40X), to calculate the percent spi using the formula (Abbas et al., 2015).

$$Percent\ sporulation(sp)inhibition = \frac{Sp\%\ of\ control - Sp\%\ of\ extract}{Sp\%\ of\ control} \times 100$$

The sporulation inhibition data were analyzed with probit analysis that cause 50% spi "IC_{50} and IC_{90} values" to calculate the exact concentration of plant extract (Monzote et al., 2014).

2.6. In-vivo anticoccidial bioassay

2.6.1. Parasite sampling and identification

The confirmed oocysts of *E. tenella* suspension and infected ceacal parts of chicken (Fig: Si-2) was obtained from poultry research institute (District: Rawalpindi, 33.438974°N, 73.043723°E) in 2% $K_2Cr_2O_7$. The oocyst suspension was filtered with fine cloth gauze, following the salt floatation method, sodium chloride salt was added to suspension till it become supersaturated, and the suspension was centrifuged at 3000 rpm for 10 min, precipitating the organic debris to harvest pure *E. tenella* oocysts and reconfirmed under microscope. The species was further identified with COCCIMORPH software (400X magnifications); the software was downloaded from website "coccidia.isb.usp.br/coccimorph". The unsporulated oocysts were settled down and separated in fresh 2% $K_2Cr_2O_7$ solution for sporulation at 21 to 32 °C to become infective in the presence of oxygen and moisture (Molan et al., 2009). The sporulating oocysts (Fig: Si-1b) were identified under microscope (Olympus-CX41) (Eassa et al., 2019).

2.6.2. Animals and treatment

One hundred and fifty "Ross 308" chicks (1 day old) were purchased from commercial hatchery (District: Rawalpindi) and reared in an animal house facility (Department of Zoology, Quaid-i-Azam University, Islamabad) provided with suitable environmental conditions. Initially, the temperature was maintained in the range of 85 to 90 °F and later on reduced by 5 °F weekly. The chicks are fed with coccidiostat-free feed and water *ad libitum*, supplemented with vitamins, and vaccinated against Newcastle and Infectious Bursal Diseases (Williams, 2006).

The *in-vivo* trial of the methanolic herbal extract was conducted which remains effective through *in-vitro* assay, for this purpose 135 healthy chicks (14 days old) were divided into nine groups each containing 15 individuals. All the groups except C1 were infected with (microtitre = 7.5×10^3 to 10×10^3 sporulated oocyst/ml) E. tenella orally. All groups were found infected after 4 to 7 days, and different parameters like weight loss, oocyst count, and diarrhea were observed to ensure the occurrence of coccidiosis. The first three groups were designated as control C1 (normal), C2 (Infected and Untreated), and C3 (Infected and SQX treated), the three infected groups (E1, E2, and E3) were treated with 5, 10, and 15 mg/ml concentration of *V. officinalis* and the groups (E4, E5 and E6) was treated with the 5, 10, and 15 mg/ml of *P. glabrum* respec-

tively (Akhtar et al., 2012). The treated groups were fed for five days from age 21 days with a dose of 1 ml/bird/day as per prescribed SQX dose (Kaingu et al., 2017).

2.6.3. Blood and tissues sample collection and processing

The blood was collected from the jugular vein of individual from each group, after then the same individuals was euthanized and dissected for tissue collection and other anatomical observations.

2.6.3.1. Hematology. The blood samples were collected in EDTA tubes from each group, thrice at the interval of 7 days i.e. 21, 28, and 35 days age, for hematological analysis like hemoglobin level, red blood cells, white blood cells, granulocytes, lymphocytes, monocytes, and platelets of all groups both post-infection and post-treatment for comparison (Campbell, 2008).

2.6.3.2. Biochemical analysis. The blood was collected in the anticoagulant-free tubes from each group and centrifuged at 2500 rpm for 15 min to obtain serum for the estimation of macromolecules at the age 21st, 28th, and 35th day. The protein content was estimated by using "Lowery protein estimation method" at the wavelength of 750 nm. The carbohydrate level of each group was assessed by the phenol sulfuric acid method at the absorption of 480 nm using a spectrophotometer (Shah et al., 2017). In addition, the liver performance of each group was analyzed by estimating AST (Aspartate Aminotransaminase) and ALT (Alanine Aminotransferase), post-infection and post-treatment using an AMP diagnostics kit at the wavelength of 340 nm, respectively with a chemistry analyzer (Motenu-MTN 658C) (Al Mathal, 2010).

2.6.3.3. Histology. To investigate the effect of methanolic extract of *V. officinalis* and *P. glabrum* on the anatomy of *E. tenella* infected groups the ceca, liver, and kidney tissues were observed both post-infection and post-treatment using a standard protocol (Deepa et al., 2020) and studied under the microscope with noticeable variations were photographed (Olympus-CX41 with Tucsen Camera) with 10X/0.25 resolution.

2.6.4. Birds performance or physical factors

The average weight of an individual from each group was measured on daily bases with the help of an electronic scale to calculate the MWG (Mean Weight Gain) post-infection and post-treatment, the post-infection and post-treatment FCR of all groups were estimated by calculating the feed consumed by individuals of each group on daily basis (Youn and Noh, 2001).

The following formulae were used to calculate MWG and FCR.

$$\mathrm{MWG} = \mathrm{MFW} - \mathrm{MIW} + \mathrm{WDB}\mathrm{FCR} = \frac{TotalFeedConsumed}{MWG}$$

MFW- Mean Final Weight, WDB- Weight of Dead Birds,

2.6.5. Fecal examination

The number of oocysts was analyzed by taking the fecal sample in both chambers of the McMaster slide with the help of a dropper after mixing 1gm of feces in 14 ml saturated sucrose solution from each group and observed at 4X magnification under the (Olympus-CX41) microscope (Zhang et al., 2013).

$$OPG = (OocystsinChamber1 + OocystsinChamber2) \times 50$$

The presence of blood in feces is one of the major clinical signs of avian coccidiosis, which shows the severity of the disease, to assess the effectiveness of methanolic plant extracts on the infected chicks bloody diarrhea was observed on daily basis with a naked eye and graded from low to high and presented in the heat map, which indicates its severity both pre and post-treatment (Habibi et al., 2016).

2.7. Molecular modeling

2.7.1. In-Silico preparation of protein target

The 3D crystallographic structure of the SAM synthetase was acquired from the Protein Drug Bank in PDB format (PDB ID: 1FUG). Prior to preparation the ligands found in protein structure were removed via PyMOL software 2.1.0 (Schrodinger, 2015), water molecules was removed to reduce interference using AutoDock Tools and polar hydrogen molecules and Kollman United Atom charges were assigned to protein molecule. The protein were then converted into the PDB format with partial charges ('Q') and AutoDock 4 atom types ('T') (PBDQT) file format and saved for later use (Trott and Olson, 2010).

2.7.2. In-silico preparation of ligand molecule

The phytocomponds of *V. officinalis* methanolic extract identified with GC–MS (Table: Si-4) were used as the ligand for molecular docking with SAM synthetase. The 3D structures of all 22 compounds were constructed and saved in.sdf format, using Chemdraw (11) then converted into PDB format via PyMOL software and minimized by computing Gasteiger charges and finally converted to PDBQT file format using AutoDock Tools (Trott and Olson, 2010).

2.7.3. Molecular docking simulation

The prepared protein and ligand were called out via Auto Dock Tools to compute suitable grid maps protein–ligands combination. During the docking process, Auto Dock 4.2 was used; the center grid parameters were set to 26.536, 17.719, and 58.288 for the x-, y-, and z-axis. The grid center was kept at 26 for x-, y-, and z-axis in angstrom with spacing of 1.00 and located at the center of the active site. A configuration file that consists of the grid box properties was created and saved as.txt file format. Then, docking was carried out using Auto Dock Vina (1.5.6) by inserting command lines in the Command Prompt application to generate the output score and the best fit model (mode 1) was selected from the 22 different conformations generated for each ligand (Trott and Olson, 2010). Interacting amino acid residues that were found in the binding site were visualized using LigPlot+ software. Amino acid residues exhibiting hydrogen bonding and hydrophobic interactions with each ligand molecule are summarized in table Si-5 (Laskowski and Swindells, 2011).

2.8. Statistical analysis

The IC_{50} concentration of aqueous and methanolic extracts of *M. arvensis, V. officinalis*, and *P. glabrum* against *E. tenella* were calculated with probit analysis to find out the exact concentration to cause 50% spi using Minitab (version 19). Further, the one-way ANOVA test was applied on percent spi, MWG, FCR, OPG (oocyst per gram), hematological and biochemical parameters to check HSD using Statistix software (version 9), and comparisons were made authentic by Tukey test to confirm the significance among the groups with $P < 0.05$ and presented in the form of means and standard deviation (Fadunsin and Ademola, 2014).

3. Results

3.1. Percent yield

The specimens are identified and submitted to the herbarium, Department of Plant Sciences, QAU and registered under voucher numbers 131454, 131,455 and 131455a respectively, shown in figure Si-1. In current study 120gm of powder of whole herb was used for extraction of each plant in methanol and water i.e. *M. arvensis*, *P. glabrum* and *V. officinalis*. The percent yield of methanolic

extracts of *M. arvensis*, *P. glabrum* and *V. officinalis* was 4.25%, 3.25%, and 2.91% respectively (Table: Si-2).

3.2. Phytochemical screening

The composition of aqueous and methanolic extracts of the respective plants illustrates the presence of a variety of the ingredients during the qualitative analysis; In addition, some compounds are observed which are common in both plants like phenols, flavonoids, alkaloids, lecoanthocynins, and couramins. The richness of both *V. officinalis* and *P. glabrum* justifies the maximum potential to inhibit *E. tenella* oocysts sporulation (Table: Si-3).

3.2.1. Fourier Transmission-Infrared spectroscopy (FT-IR)

The FTIR spectra of all three herbs show several bands in the wavelength range 4000–500 cm^{-1} and most of them are common in all three with the exception that more bands appeared in *V. officinalis* (Fig: Si-3a). Each wave number was studied in detail with reference ranges in the literature (Pavia et al., 2003). The region of the broad absorption band at 3550–3000 cm^{-1} was observed in all the respective herbal extracts which might be assigned to the presence of an alcoholic or phenolic group; the broadness of the band might be attributed to the presence of possible hydrogen bonding. It is further supported by another common absorptions band at 1113 cm^{-1} which indicates the presence of C–O functionality. At the same time; we cannot neglect the possible presence of the amine group as well, as its absorption band also appears above 3100 cm^{-1} which is again present in all three herbs and might have been overlapped by the O—H broad absorption. The C—N absorption band also appears in almost the same region in which C—O absorbs. All herbs that share an absorption band below 3000 cm^{-1} may be assigned to the C–H sp^3 absorption. The band at above 2500 cm^{-1} may be attributed to the presence of thiols (S–H) functionality. The absorption band appearing at 1660 cm^{-1} may be due to the presence of C═O or C═N moiety. Absorption wave bands at 1440 and 1414 cm^{-1} may be assigned to the —CH_2– bending. The wavelength absorption band at 1020 cm^{-1} may be due to the C—F stretch and the final absorption wavelength band near 600 cm^{-1} may be due to the C—Cl or C—I stretch (Fig: Si-3b & Si-3c).

3.2.2. Ultra Violet visible spectroscopy (UV–Vis)

The UV–Vis analysis was performed for the identification of the phytochemical composition of *V. officinalis, P. glabrum,* and *M. arvensis,* to identify the compounds with σ & π bonds, lone pair of electrons, chromophores, and aromatic rings. The qualitative spectrum was selected on the bases of sharp peaks and proper baseline falling in the range of 200 to 800 nm wavelength. The UV–Vis profile of *V. officinalis* methanolic extract was observed in the range of wavelength of 200 to 800 nm. The profile shows various peaks at 226.13, 289.68, 332.71, 410.11, and 665.29 nm with the absorption of 3.78, 1.52, 1.82, 0.54, and 0.18 respectively. It shows the indication of hetero atoms (S, N, and O), Unsaturated alkenes in the UV region with π-π* transition, and carbonyls in the visible region with n-π* transition. The last peak of 665.29 nm shows the presence of N = O chromophores (Fig: Si-4a). The profile of *P. glabrum* shows the peak of 395 nm with an absorption of 2.4 in the UV region, which indicates the presence of aromatic compounds with π-π* transition, and the rest of the two peaks in the visible region at 610 and 670 nm with the absorption of 0.18 and 0.54 respectively, which indicates the presence of carbonyls with n-π* transition (Fig: Si-4b). The UV–Vis profile of *M. arvensis* shows peaks with n-π* and π-π* transition at 270, 320, 410, 615, and 670 nm with the absorption of 1.1, 0.8, 1.6, 0.3, and 0.68 respectively, which indicates the presence of unsaturated alkenes, aromatic compounds, and carbonyls (Fig: Si-4c).

3.2.3. GC–MS of V. officinalis

The GC chromatogram (Fig. 1) of methanolic herbal extracts of *V. officinalis* specified twenty one peaks, which refers to the presence of organic compounds. GC–MS is suitable technique used for detection of volatile compound, includes alkanes (Aliphatic hydrocarbons), Carboxylic acid, phenol, esters, ether and triterpenes. Retention time (minutes), percent peak area, molecular formula, molecular weight can be used for identification of phytochemical components. Out of the whole compound detected 7 compounds area present in abundance covering high peak area percentage i.e. Hexadecane (4.84%), Sulfurous acid, Octadecane (3.34%), Squalene (4.75%), Hexacosane (8.11%), Octacosane (8.47%), Tetratetracontane (5.95%), and Pentacosane (14.18%) are reported as a major constituents in methanolic herbal extract of *V. officinalis* whereas the rest of compounds mention in table (Si-4) are present in minor quantity.

3.3. In-vitro bioassay (Antioxidant potential and sporulation inhibition assay)

As for the biochemical composition of plant extracts concerned, the SOD, GSH, and Protein contents were estimated. The level SOD is recorded high in *V. officinalis* than *P. glabrum* and *M. arvensis*. Similarly, GSH level in *V. officinalis* extract is also recorded higher and both *P. glabrum* and *M. arvensis* have the same amount of GSH (Fig: Si-5). The amount of protein content is almost the same in *V. officinalis* and *P. glabrum*, whereas *M. arvensis* does not have a sufficient amount of protein.

The unsporulated oocysts was used for *in-vitro* spi-assay, whereas in the case of *in-vivo* trial the oocyst was kept at room temperature (21 to 32 °C), in 2% $K_2Cr_2O_7$ in the presence of oxygen and observed after 24 h under a microscope, the maximum number of the oocyst were sporulated and able to cause infection in the chicks. After extraction process all selected plants yield into 4 to 5 g of crude extract in methanol and aqueous solvent, which was further used for anticoccidial activity.

In the current study the higher spi rate was observed in the case of methanolic extracts of *V. officinalis* at an incubation period of 48 h is 81.04 ± 0.96 % greater than that of *P. glabrum* (72.47 ± 2.6 5 %) and at the same period, the inhibition rate of *M. arvensis* was<50%. Similarly, the aqueous extract at the same incubation period has somewhat similar but lower inhibition rate. Subjecting the oocysts to the same herbal extracts for some more time and the inhibition percent was observed again after 72 h incubation period and it was noted that overall the effectiveness of both methanolic and aqueous extracts is decreased by 5 ± 1 % (Fig: Si-6a, b, c & d). Whereas, mint (*M. arvensis*) has<50% inhibitory affect against *E. tenella* oocysts (Fig: Si-6e & Si-6f). Further, the common rising trend in spi of oocysts was noticed with increase in concentration. The $K_2Cr_2O_7$ and SQX treated groups were run as a control for comparative analysis. To analyze the potential of the selected plant extracts, the IC_{50} was estimated with probit analysis, which showed activity of *V. officinalis* at 48 h in both methanolic and aqueous extracts shown in Table 1, Though *P. glabrum* interrupted the sporulation process but the rate of inhibition was found low than that of *V. officinalis* extract. The inhibitory activity of *M. arvensis* is not remarkable so that's why the IC_{50} value signals that much amount of crude extract is required to inhibit oocyst sporulation.

3.3.1. Hematological study

The effect of methanolic plant extracts of *V. officinalis* and *P. glabrum* on the blood composition of all chicks groups was investigated using different hematological parameters (Hb, RBCs, WBCs,

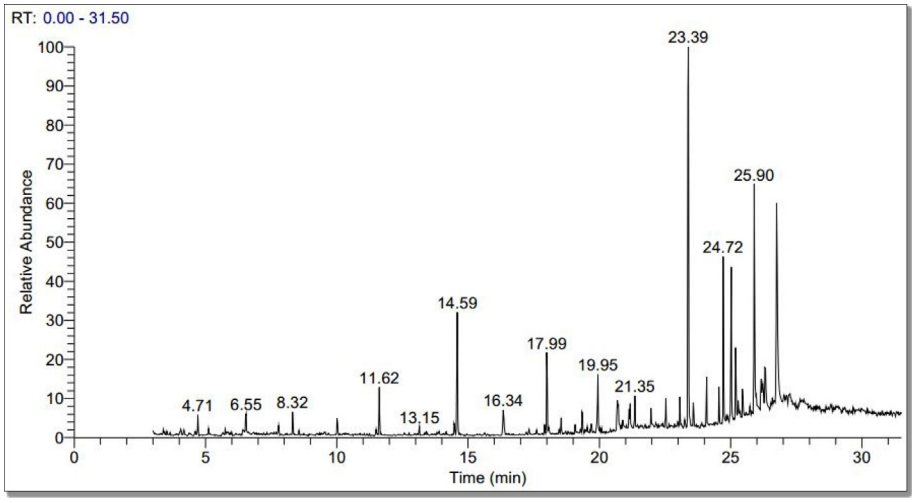

Fig. 1. GC_MS chromatogram of methanolic crude extract of *V. officinalis.*

Table 1
Inhibitory concentration (IC_{50}) of aqueous and methanolic plant extracts against *Eimeria* sp. oocysts.

Incubation period	Plant	Extract	Mean	IC_{50} (mg/mL) (UCL-LCL)	IC_{90}(mg/mL) (UCL-LCL)	X^2 (*df* = 3)
48 h	*V. officinalis*	A	6.68 ± 1.16	6.68 (8.81–4.18)	44.99 (51.37–40.27)	31.11
		M	0.14 ± 1.55	0.14 (2.87–−3.36)	39.53 (45.56–35.15)	33.41
	P. glabrum	A	15.46 ± 1.05	15.46 (17.5–13.37)	55.92 (63.93–50.01)	31.03
		M	12.08 ± 1.05	12.08 (14.12–9.92)	51.79 (59.15–46.35)	23.52
	M. arvensis	A	39.19 ± 2.12	39.19 (44.03–35.5)	80.60 (93.11–71.54)	40.49
		M	36.73 ± 1.98	36.73 (41.24–33.2)	78.87 (91.17–69.97)	39.86
72 h	*V. officinalis*	A	16.3 ± 0.93	16.3 (18.17–14.48)	51.35 (57.6–46.5)	32.3
		M	13.13 ± 0.90	13.13 (14.9–11.3)	47.28 (52.99–42.9)	31.5
	P. glabrum	A	22.26 ± 1.27	22.26 (24.9–19.9)	66.26 (76.68–58.74)	30.38
		M	18.46 ± 1.07	18.46 (20.6–16.4)	58.37 (66.52–52.22)	21.41
	M. arvensis	A	44.37 ± 2.15	44.37 (49.2–40.61)	79.37 (90.09–71.41)	14.97
		M	39.85 ± 1.84	39.85 (43.9–36.61)	74.89 (84.52–67.62)	18.27

Platelets, Lymphocytes, Monocytes, and Granulocytes). The mean values of different hematological parameters of the groups E2 and E6 were found in the normal range just like the uninfected and untreated (C1) and SQX treated group (C3), with $P < 0.05$, Hb

Table 2
The hematological profile of different broiler groups at age day 21, 28 and 35.

Hematology Parameters		Control groups			*V. officinalis*			*P. glabrum*		
		C1	**C2**	**C3**	**E1**	**E2**	**E3**	**E4**	**E5**	**E6**
Hb	7th dpi	8.7 ± 0.7	7.6 ± 0.3	7.5 ± 0.5	7.8 ± 0.2	7.7 ± 0.1	7.8 ± 0.3	7.7 ± 0.4	7.9 ± 0.4	7.4 ± 0.2
(g/dl)	7th dpt	10.6 ± 0.42	7.8 ± 0.9	9.2 ± 0.2	7.9 ± 0.3	9.6 ± 0.5	8.1 ± 0.3	7.8 ± 0.5	8 ± 0.4	9.6 ± 0.4
	14th dpt	13.1 ± 0.2	7.4 ± 0.2	12.1 ± 0.6	7.7 ± 0.1	10.8 ± 0.3	8.6 ± 0.1	7.5 ± 0.2	8.1 ± 0.2	10.1 ± 0.4
RBC	7th dpi	1.8 ± 0.07	1.62 ± 0.05	1.6 ± 0.06	1.7 ± 0.14	1.6 ± 0.1	1.5 ± 0.06	1.4 ± 0.1	1.6 ± 0.02	1.59 ± 0.03
($\times 10^6$/µl)	7th dpt	2.2 ± 0.15	1.57 ± 0.08	1.7 ± 0.1	1.3 ± 0.28	1.8 ± 0.14	1.4 ± 0.2	1.5 ± 0.08	1.7 ± 0.05	1.66 ± 0.06
	14th dpt	2.9 ± 0.02	1.5 ± 0.02	2.4 ± 0.4	1.7 ± 0.26	2.3 ± 0.4	1.9 ± 0.08	1.4 ± 0.3	1.8 ± 0.06	2.03 ± 0.06
WBC (/µl)	7th dpi	8046 ± 28	8875 ± 36	8877 ± 18	8868 ± 78	8341 ± 1	8916±	8110 ± 15	8228 ± 16	8296 ± 8
	7th dpt	9098 ± 42	8243 ± 9	9003 ± 28	8069 ± 49	9051 ± 15	8199 ± 48	8447 ± 42	8545 ± 62	8987 ± 51
	14th dpt	10443 ± 9	7705 ± 27	10020 ± 39	7998 ± 25	10151 ± 17	9029 ± 26	8108 ± 29	8832 ± 28	9795 ± 10
Platelets ($\times 10^3$/µl)	7th dpi	440 ± 1.1	470 ± 1.8	467 ± 3.3	476 ± 06	479 ± 0.9	466 ± 1.4	477 ± 0.9	465 ± 1.9	456 ± 0.3
	7th dpt	435 ± 3.5	461 ± 0.8	461 ± 4.1	470 ± 0.7	481 ± 0.6	463 ± 3.0	473 ± 0.6	478 ± 0.2	467 ± 0.5
	14th dpt	462 ± 2.8	453 ± 0.6	530 ± 4.4	460 ± 1.0	497 ± 0.8	479 ± 0.9	466 ± 0.9	484 ± 0.5	486 ± 0.6
Lymphocytes (%)	7th dpi	64.9 ± 1.4	77.5 ± 0.67	77 ± 0.3	75.5 ± 0.5	77 ± 0.5	77 ± 0.9	75 ± 0.2	76.7 ± 0.4	77.3 ± 0.5
	7th dpt	65.6 ± 0.93	86 ± 0.7	73 ± 0.7	79.8 ± 0.6	74 ± 0.4	83 ± 0.6	79 ± 0.9	78.6 ± 0.7	75.7 ± 0.5
	14th dpt	66.2 ± 1.05	91.5 ± 1.1	72 ± 1.1	83.6 ± 1.2	72 ± 0.1	80 ± 0.9	85 ± 0.8	82.3 ± 0.7	73.8 ± 0.3
Monocytes (%)	7th dpi	15.8 ± 0.4	22 ± 1.5	22.8 ± 0.5	20.8 ± 0.4	21 ± 0.6	21.2 ± 0.8	23.5 ± 0.6	22.7 ± 0.6	22 ± 0.9
	7th dpt	15.7 ± 0.8	18 ± 0.4	23 ± 0.4	18.9 ± 0.4	16.6 ± 0.4	16.6 ± 0.4	20.7 ± 0.4	18.9 ± 0.3	22.7 ± 0.5
	14th dpt	15.6 ± 0.7	15 ± 0.6	27.6 ± 0.3	14.5 ± 0.5	19.6 ± 0.9	19.6 ± 0.8	15.8 ± 0.8	18.2 ± 0.3	25.9 ± 0.9
Granulocytes (%)	7th dpi	16 ± 0.4	18 ± 0.3	16.7 ± 0.5	16.9 ± 0.4	16 ± 0.5	16.9 ± 0.6	17.4 ± 0.5	17.9 ± 0.5	17 ± 0.4
	7th dpt	16.9 ± 0.8	20 ± 0.7	13.1 ± 0.2	19 ± 0.8	14 ± 0.3	19 ± 0.5	19.8 ± 0.4	19.3 ± 0.3	15 ± 0.4
	14th dpt	17.9 ± 0.8	21.2 ± 1.5	11.1 ± 0.2	22 ± 0.7	11.9 ± 0.2	16.3 ± 0.5	22.9 ± 0.3	22.1 ± 0.3	13.7 ± 0.4

C1- no-infection and Untreated; C2– Infected and Untreated; C3– Infected and treated with Sulfaquinoxaline; E1- Infected and treated with *V. officinalis* (5 mg/ml); E2- Infected and treated with *V. officinalis* (10 mg/ml); E3- Infected and treated with *V. officinalis* (15 mg/ml); E4- Infected and treated with *P. glabrum* (5 mg/ml); E5- Infected and treated with *P. glabrum* (10 mg/ml); E6- Infected and treated with *P. glabrum* (15 mg/ml), (dpi- day post infection; dpt- day post treatment).

level, RBCs, WBCs, platelets and monocytes count of groups E2 and E6 shows an increase with increase in age, similar to the normal uninfected chicks. In contrast, the level of lymphocytes and granulocytes drops with the passing age, similar patterns were noticed in C3 group. The hematological outcome of the studied groups shows a positive impact of the respective concentrations on the methanolic extracts (Table 2).

3.3.2. Biochemical study of serum

The biochemistry of all groups of chicks was examined by determining the quantity of serum protein, carbohydrate, ALT, and AST at the intervals of 7 days at 21st, 28th, and 35th day. The quantity of protein was 3.17 ± 0.03, 3.22 ± 0.03, and 3.21 ± 0.02 g/l (Fig: Si-7a) and level of carbohydrates in the uninfected control group (C1) was 308 ± 0.007, 292 ± 0.01 and 302.4 ± 0.01 mg/ml at day 21, 28 and 35 age respectively, remains normal throughout the trial(Fig: Si-7b). In contrast, the infected groups were found to have lower level of these macromolecules initially but after the treatment with the methanolic crude extracts of *V. officinalis* and *P. glabrum,* some groups showed a continuous increase in protein level i.e. E2 treated with *V. officinalis* (10 mg/ml) has 0.97 ± 0.01, 1.96 ± 0.02 and 3.0 6 ± 0.02 g/l protein contents, E3 treated with *V. officinalis* (15 mg/ml) has 0.98 ± 0.03, 2.11 ± 0.01 and 3.85 ± 0.04 g/l and group E6 treated with *P. glabrum* (15 mg/ml) was recorded with increasing level of protein i.e. 1.05 ± 0.02, 2.06 ± 0.1 and 3.90 ± 0. 04 g/l over time. Similarly, the level of carbohydrates of *V. officinalis* and *P. glabrum* treated groups were significant and similar to that of the uninfected group i.e. groups E2 has 272 ± 0.01 mg/ml and E6 had 278 ± 0.1 mg/ml at the age of 35 days respectively.

Similarly, the amount of ALT and AST was lowered in the uninfected and higher in the infected groups. The level of both enzymes decreased in the extract-treated groups, whereas the untreated group (C2) showed a continuous increase in mean ALT and AST levels. The ALT level of group E2 treated with *V. officinalis* (10 mg/ml) crude methanolic extract was 165 ± 0.08, 124 ± 0.07, and 107 ± 0.03U/L and the group E6 treated with *P. glabrum* (15 mg/ml) crude methanolic extract was found 165 ± 0.3, 84 ± 0.05, and 80 ± 0.05U/L at the age of day 21, 28 and 35 respectively (Fig: Si-7c). The AST level of group E2 treated with *V. officinalis* (10 mg/ml) crude methanolic extract was 97.54 ± 0.03, 87.99 ± 0.02 and 31.14 ± 0.01U/L and E6 treated with methanolic crude extract of *P. glabrum* (15 mg/ml) was found 81.42 ± 0.02, 44.81 ± 0.03 and 31.85 ± 0.01U/L at the age of day 21, 28 and 35 respectively (Fig: Si-7d).

3.3.3. Histology

A comprehensive histo-pathological examination of all groups was performed. The ceacal, liver and kidney tissues were stained to observe the changes. The remarkable changes in the histomicrographs of the said tissues were noticed, which illustrates the recovery of individuals of different groups treated with *V. officinalis* (Fig. 2b) and *P. glabrum* (Fig. 2c) in comparison with the untreated and *E. tenella* infected group (Fig: 9a). The histological micrographs (Fig. 2a) of the control C1 group shows normal morphology as it remained uninfected, whereas the morphology of C2′s liver is altered having compromised sinusoidal space, and the distribution of hepatocytes are changed as compared to C1, while the observation of kidney tissues demonstrates the shrunken glomerulus, degenerative collecting ducts, vacuole formation and increased bowman space, all these observations ensure the presence of parasitism. The protective groups i.e. C3 which is treated with SQX show a recovery of sinusoidal spaces in liver tissues and lowering of spaces observed between hepatocytes. The glomerulus and tubules morphology in the kidney tissues also show recovery as compared to the control C1 group (C: ceca, L: liver, and K: kidney). As for the experimental groups concerned which were treated with various dilutions of methanolic extracts of *V. officinalis* and *P. glabrum*, the histological study of the *V. officinalis* treated tissues was present in Fig. 2b, which shows that chicks of group E1, treated with lower concentration (5 mg/ml) of the extract, was not recovered by forming vacuole in kidney tissues, the appearance of sinusoidal spaces in liver and degeneration of cecum tissues (Fig. 2b). The individuals of groups E2 and E3, which has improved FCR and maximum weight gain by treatment with *V. officinalis* methanolic extract (10 & 15 mg/ml) respectively (Table 3), the reason is that the histological observations of the respective group illustrate recovery from coccidiosis in the form of no vacuoles in kidney tissues and no such apparent degeneration in liver and cecum tissues. The histomicrographs of the various tissues (Fig. 2c) of group E4 individuals has shown no recovery in all three types of tissues, after treatment with 5 mg/ml methanolic extract of *P. glabrum*. Whereas the kidney and liver tissues recovered by treatment with 10 and 15 mg/ml, appeared as no degeneration in tissue structure, and no vacuole formation with exception of a small amount of disruption in cecum and kidney tissues of group E6.

3.4. Mean weight gain and feed conversion ratio

The two basic parameters of MWG and FCR of all experimental groups were calculated thrice at different ages i.e. 21st, 28th, and 35th day age with the interval of 7 days. The summary of the average weight gain showed the medium concentration (10 mg/ml) of *V. officinalis* and a high dose (15 mg/ml) of *P. glabrum* were found effective against *E. tenella* oocysts sporulation and the individuals were found with enough mean weight gain. Adding to it, the MWG of the chicks as a result of the effective concentration "E2" of *V. officinalis* was 290 ± 30.3, 313 ± 22.8, and 621 ± 6.9gm MWG, whereas, group E6 treated with P. glabrum (15 mg/ml) was noticed with 250 ± 16, 292 ± 11 and 513 ± 10.5gm MWG at day 21, 28 and 35 respectively, the results show direct relation between MWG and time after drug administration i.e. with the passage of time the MWG of the chicks increases. In the present *in-vivo* study, the detail analysis of MWG of different experimented groups confirms the effectiveness of *V. officinalis* methanolic extract as compared to *P. glabrum* crude extract. The activity of both plant extracts were assured by comparing the MWG of the respective treated groups with SQX treated control group (C3) i.e. 259 ± 17.6, 437 ± 10.3, and 534 ± 6.3gm at the respective age, which is almost near to the MWG of E2 and E6. In short, the booster effect of methanolic crude extracts of both plants resulted in maximum weight gain with growing age, without harming the chicks (Table 3).

3.5. Fecal oocysts count and diarrhea grading

The OPG of each group was counted under microscope using the McMaster chamber on daily basis till the end of the experiment. In all infected groups, the number of oocysts was decreased after oral treatment with methanolic crude extract of V. officinalis and P. glabrum to the chicks. The uninfected group "C1" remained negative for *E. tenella* oocysts, while the fecal sample of group C2 was found with a huge number of oocyst i.e. 21,850 OPG, C3 showed 5250 OPG at age of 18 days and raised to 10,300 OPG at 23 days age and after treatment with SQX, it dropped to 3100 OPG at the age of 35 days. The group (E2) treated with *V. officinalis* (10 mg/ml) was diagnosed with 6950 OPG at the age of 17 days and rises to a peak level of 10,900 OPG at day 25 age, but when the respective methanolic extract came into action, the OPG dropped to 3950 oocysts. Similarly, the group E6 was initially reported with 5150 OPG at 18 day age and gradually raised to 12,750 OPG at 27 day age and after treatment with *P. glabrum* (15 mg/ml), the OPG

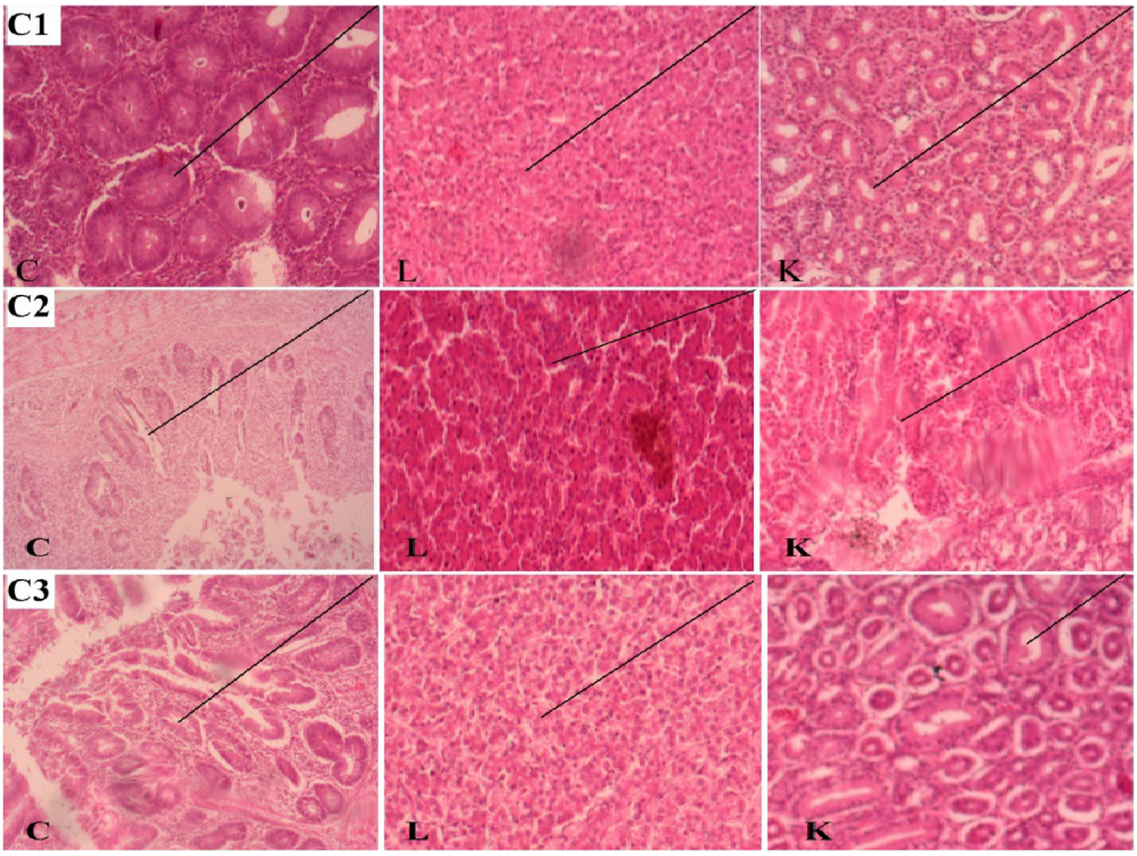

Fig. 2a. Photomicrograph of the histological study of the control groups (C. ceca, L. liver and K. kidney).

dropped to 5700 oocysts at 35 day age. In short, the methanolic crude extract of both plants has an inhibitory role in the sporulation of E. tenella oocysts but V. officinalis was found more potent with low number of OPG at the end of trial (Fig. 3).

Bloody diarrhea appeared within 7 days in all the E. tenella infected chicks' groups after oral infection. The appearance of diarrhea varied in different groups and even reached the severe level, but after the treatment on day 21 age with the respective concentrations and the severity of coccidiosis decreased due to its healing potential i.e. E2 and E3 treated with (medium and high concentration) *V. officinalis* recovered at 6th day of treatment, SQX treated group recovered at 7th day & *P. glabrum* treated groups took 9 days to recover from diarrhea after treatment and ultimately bloody diarrhea slowly disappeared with the healing potential of methanolic extracts of V. officinalis and P. glabrum (Fig. 4).

3.6. Molecular modeling

All 22 ligands isolated from methanolic extract of *V. officinalis* were docked with SAM synthetase. The binding affinities between of different ligands with target protein show different score values. The lower will be the binding score; the higher will the binding affinity. The binding score values of ligands greater than − 5.0 kcal/mol were excluded. Among all protein/receptor–ligand pairs, only four ligands showed the lowest binding energy ranging from − 5.0 to −6.4 kcal/mol (Table: Si-5). The hydrophobic interactions in 2D structures generated via LigPlot+ (Fig. 5a-d), shows the involvement of relatively varied number of interacting amino acid residues across the different ligand–receptor complexes. These interactions enhance the binding affinity and biological activity of the complex molecules and help in stabilizing the biochemical environments of target-drug complexes. The hydrophobic interaction of ligands with amino acid residues of SAM synthetase are demonstrated in table Si-5. Among all four ligands, Strychane, 1-acetyl-20a-hydroxy-16-methylene demonstrated the best linkage with lowest binding score of −6.4 Kcal/mol (Fig. 5a).

4. Discussion

Eimeria species are deadly harmful to the poultry industry and lead to millions of dollars loss annually. To overcome this issue veterinary sector conducted many trials, some of them worked but most leads to making the parasite more resistant. The current study is conducted to check the effect of natural, economic, and easily available products or flora, which recommends that a higher rate of spi were recorded at a higher concentration of methanolic extract of *V. officinalis* with IC_{50} 0.14 mg/mL against *E. tenella* at 48 h incubation. Further, this plant was found rich in ingredients as shown in the qualitative phytochemical analysis. *P. glabrum* also showed more than 50% activity at both medium and higher concentrations.

The constituents derived from *V. officinalis* have an impressive effect on the prevention of cancer in a human colon cell line with IC_{50} < 20 mg/mL (Encalada et al., 2015). In humans, it helps in the regulation of lactation and menstrual cycle (Hernandez et al., 2000), and is also used in curing rheumatism and joint pain (Li et al., 2003). In edema, it shows effective results in comparison with marketed ointment peroxicam (Calvo, 2006). The oil derived from *V. officinalis* can cause cell death in the case of chronic lymphocytic leukemia (Martino et al., 2011). The number of medicinally important herbs with 70 to 100 % efficacy against *E. tenella*, which concludes that *Sophora flavescens* to cure the life-threatening avian infection coccidiosis (Youn and Noh, 2001),

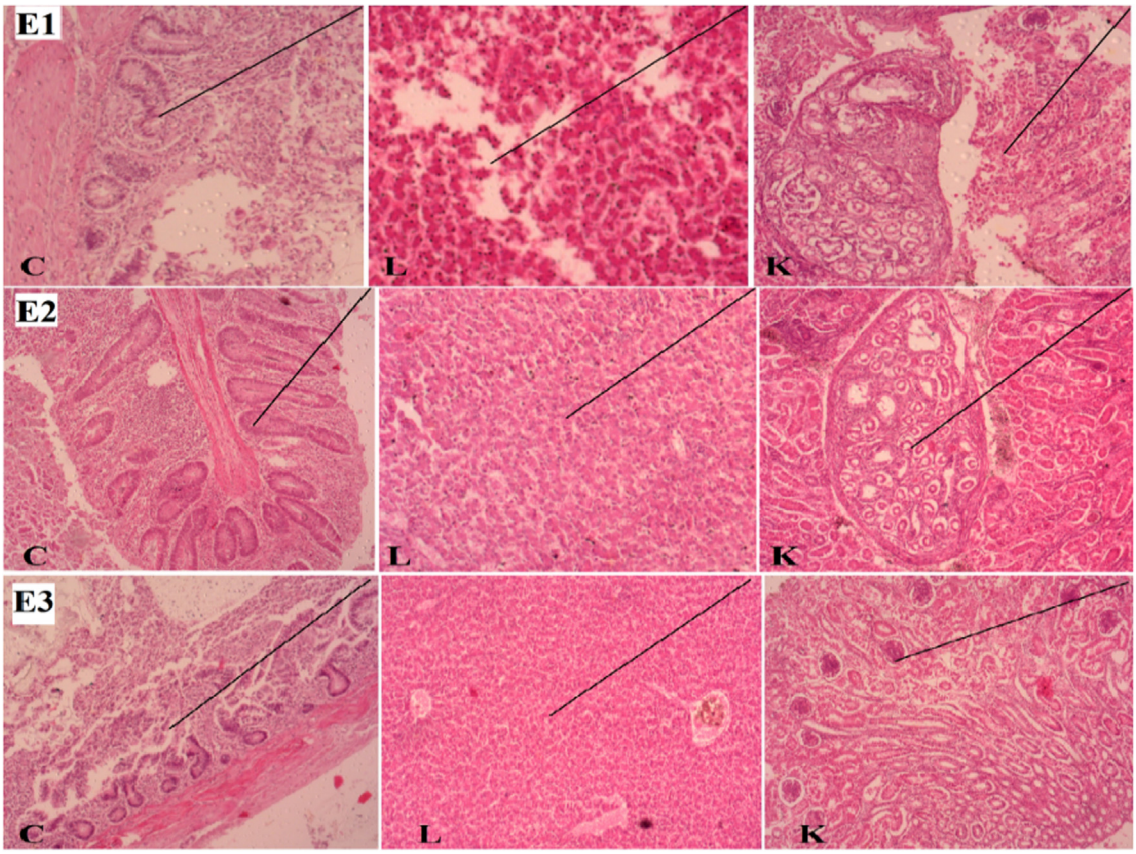

Fig. 2b. Photomicrograph of the histological study of the *V. officinalis* (C. ceca, L. liver and K. kidney).

Punica granatum, commonly known Pomegranate is effective against *Eimeria spp*. by lowering its population up to 50% in the infected flock (Dkhil, 2013). Artemisinin derived from *Artemisia sieberi* is capable to decrease the quantity of oocyst in chicks infected with *E. tenella* and *E. acervulina* (Arab et al., 2006). Methanolic extract of *V. officinalis* extract has shown round about 30% activity against fungus species *Rhizopus stolonifer* and *Penicillium expansum* (Casanova et al., 2008). It shows culicidal activity with LC_{50} of 38 ppm (Pavela *et al*., 2009). The ethanolic content of *V. officinalis* stem is effective against *Staphylococcus aureus* (Ahmad *et al*., 2012). The commercially available drug apacox was tested against *E. tenella* with better results like lowering of diarrhea and good feed conversion rate (Christaki et al., 2004). SOD is the enzymes that can help in the conversion of superoxide anion to hydrogen peroxide (Sheng et al., 2014), and the peroxide species like H_2O_2 are found to be effective against coccidian oocysts by deactivating or arresting it from further development (Lee and Lee, 2007), this statement provides the evidence that the plant extract or drug having more quantity of SOD will effective against parasite population, and in current study *V. officinalis* has a high level of SOD than other plants (Fig: Si-5) and that's why it has shown much inhibition against *E. tenella* (Figure: Si-7a & Si-7b). GSH commonly known as glutathione is the reducing form of glutathione, it acts as a neutralizer in a cell by neutralizing oxidants molecules, and in turn, it detoxifies the harmful effect of various compounds, it has shown in the study (Li et al., 2010) that S-nitroso glutathione, actually a derivative of glutathione has an inhibitory effect on *Eimeria* spp. and can interrupt the sporulation process of the parasite, which may keep the *Eimeria* oocysts in arrest stage. So it is concluded from figure (Si-2), that a high level of glutathione will have a more inhibitory effect and vice versa. The asymptomatic coccidiosis in chicks interrupts many anabolic activities, which ultimately lead to lesser protein production and as a result, it disturbs the performance of chicks (Mathew et al., 2000). The protein content in the diet or drug can have a positive impact on the live performance of chicks (Lee et al., 2011), which in turn makes it healthy in the form of improved immunity. In the current study, the *V. officinalis* extract has a sufficient amount of protein contents which can upregulate the chicks performance as compared to *P. glabrum* and *M. arvensis*. The results demonstrate the higher inhibition activity of *V. officinalis* and comparative of all three plant extractives shows a minute difference between methanolic and aqueous extracts, which means that methanol extractives have some extra ingredients which enhance their inhibition rate against *Eimeria* oocyst. As for the *in-vivo* trial concerned, several medicinal herbs are reported to control coccidiosis from poultry; some common plants used against different *Eimeria* spp. are *Sophora flavescens* with 70% anticoccidial efficacy (Youn and Noh, 2001), *Punica granatum* commonly known as Pomegranate showed 50% effectiveness (Dkhil, 2013). The inhibitory activity of *Azadirachta indica, Nicotiana tobaccum, Trachyspermum ammi*, and *Calotrpois procera* has good results against sporulating oocysts in comparison with amprolium (Zaman et al., 2012). *Artemisia sieberi* (artimisinin) *is* capable to decrease the *E. tenella* and *E. acervulina* oocyst quantity (Arab et al., 2006).

In general, the *Eimeria spp* affected hosts has lowered MWG as compared to the healthy one, due to the disturbed nurturing or metabolism (Orengo et al., 2012), Similarly the current study showed the lowered protein and carbohydrates contents of the infected chicks, the probable reason for this decline is the disruption of the epithelial lining of the intestine, which is unable to assimilate sufficient amount of nutrients and hence leads to weight loss. Further, this statement also supports the recovery of chicks treated with *V. officinalis* and *P. glabrum*, which is reflected in the form of good MWG, high quantity of proteins and carbohydrates,

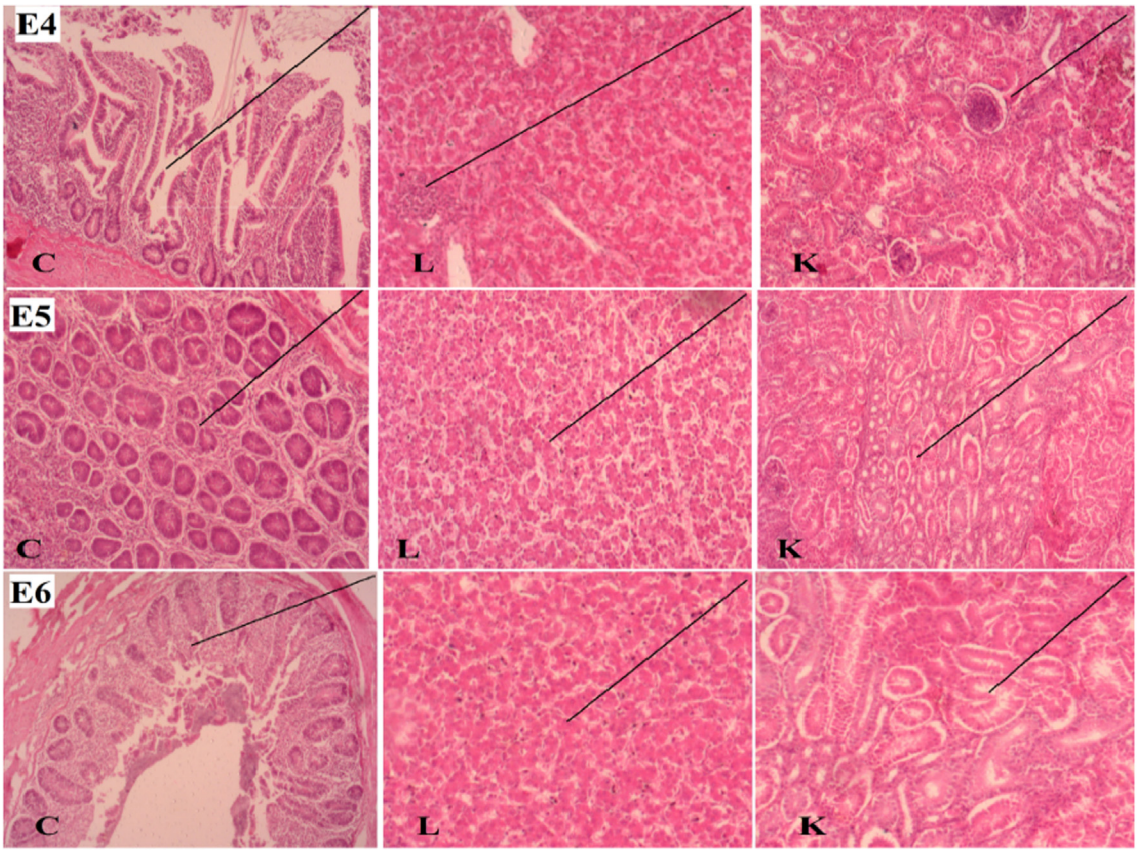

Fig. 2c. Photomicrograph of the histological study of the *P. glabrum* (C. ceca, L. liver and K. kidney).

Table 3
The mean weight gain (gm) and feed conversion ratio of broilers groups at age day 21 and 28 & 35.

Parameter		**Control groups**			*V. officinalis*			***P. glabrum***		
		C1	C2	C3	E1	E2	E3	E4	E5	E6
MWG	7th dpi	435 ± 18^{a}	242 ± 15^{b}	259 ± 17.6^{b}	270 ± 9.5^{b}	290 ± 30.3^{b}	283 ± 15.6^{b}	270 ± 27.5^{b}	264 ± 1.6^{b}	250 ± 16^{b}
	7th dpt	525 ± 10^{a}	185 ± 9.6^{e}	437 ± 10.3^{b}	316 ± 24.7 ^{cd}	313 ± 22.8^{c}	327 ± 17.5^{c}	201 ± 19^{e}	199 ± 16^{e}	292 ± 11^{d}
	14th dpt	700 ± 23^{a}	143 ± 1.5 ^{g}	534 ± 6.3^{b}	293 ± 67.9^{e}	621 ± 6.9^{bc}	493 ± 84 ^{cd}	278 ± 25^{f}	439 ± 65.2^{e}	513 ± 10.5^{d}
FCR	7th dpi	1.15 ± 0.02^{f}	2.14 ± 0.09 ^{cd}	1.87 ± 0.07^{e}	1.98 ± 0.04^{de}	2.67 ± 0.09^{ab}	1.99 ± 0.02^{de}	2.79 ± 0.19^{a}	2.39 ± 0.02^{bc}	2.09 ± 0.12^{de}
	7th dpt	1.27 ± 0.01^{f}	2.59 ± 0.04^{a}	1.5 ± 0.02^{e}	1.72 ± 0.03^{de}	1.95 ± 0.11 ^{cd}	1.57 ± 0.07^{e}	2.22 ± 0.16^{b}	2.07 ± 0.08^{bc}	1.88 ± 0.08 ^{cd}
	14th dpt	1.29 ± 0.04^{f}	3.07 ± 0.08^{a}	1.87 ± 0.02^{d}	2.29 ± 0.02^{c}	1.95 ± 0.02^{d}	1.69 ± 0.06^{e}	2.79 ± 0.05^{b}	2.00 ± 0.03^{d}	1.71 ±.04^{e}

(dpi- day post infection, dpt- day post treatment; As superscript of both treated and untreated groups are different showing significant differences, denotes the tukey test variables).

and histomicrographs of the ceacal part of the respective groups (Morris and Gasser, 2006). The disturbed assimilation process leads to imbalanced hematology and biochemical profile in different infected groups at the 7th day post-infection. The phenomenon of weight loss or stunted growth was controlled by conducting the bioactivity of ethanolic crude extracts of *V. officinalis* and *P. glabrum* against *E. tenella* due to its medicinal importance as reported in earlier research. Further, the naturally derived compounds can augment the immunity of the host (Freier et al., 2003). The significant weight gain and other parameters of C1 broilers elucidate the sterility of the environment provided to the chicks (Orengo et al., 2012). Table: Si-3 shows the richness of both plants (*V. officinalis and P. glabrum*), whereas the GC report of methanolic extract of V. officinalis shows the presence of bioactive phyto-components therefore the Freier and coworkers (2003) states that, naturally derived compounds boost the immune response to parasitic infection. The effective concentrations of *V. officinalis* (10 mg/ml) and *P. glabrum* (15 mg/ml) results in good weight gain of chicks' just next to the SQX treated control group, especially in group E2 and E6, similar findings were also reported by Kaingu and colleagues in 2017, in which maximum weight was gained by chicks after treatment with *Aloe secundiflora* extract, his findings supports the phenomenon of slow growth by group C2, which is remained infected and untreated. The FCR of groups E1, E4, and E5 was recorded above 2.00 and C2 was a poor FCR of 3.07, whereas the groups E2 and E6 and controls "C1 and C3" has FCR values in the normal range with normal hematology (Table 3) and biochemical profile (Fig. 5a). Similarly, Naidoo and coworkers (2008) reported the activity *Tulbaghia violacea*, *Vitis vinifera*, and *Artemisia afra extracts* and toltrazuril treated chicks have good FCR as compared to infected chicks. The methanolic crude extracts of *V. officinalis* and *P. glabrum* exhibit antioxidant activity (Casanova et al., 2008; Raja and Ramya, 2017), which supports the reduction in oocysts shedding in the effective groups. The natural products usually don't harm a living being with some exceptions, as El Banna, (2016) said that *Moringa olifera* doesn't lead to any toxic effect. Similarly, the chicks under trial don't bear any negative pressure of *V. officinalis* and *P. glabrum* methanolic extracts except for the

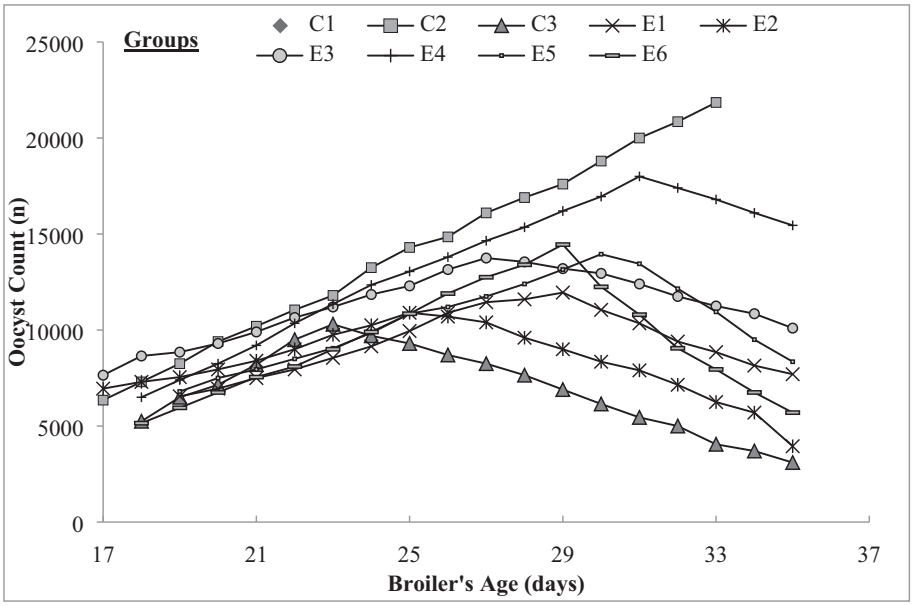

Fig. 3. The oocyst count (OPG) of *E. tenella* diagnosed in various broiler groups.

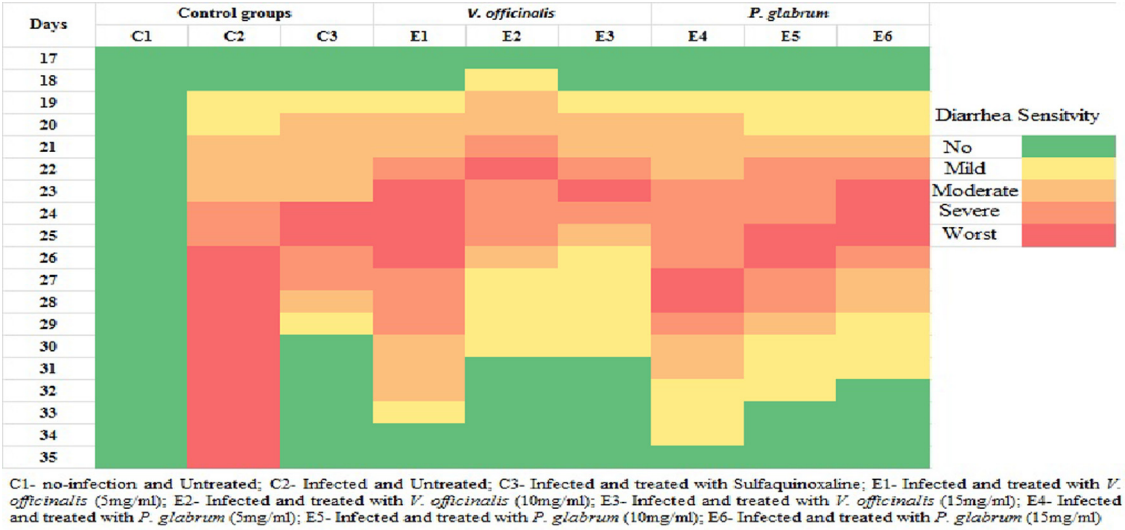

Fig. 4. The heat map shows the severity level of bloody diarrhea after *E. tenella* infection and post treatment of infected groups with respective plant extract till the end of experiment.

higher concentration (15 mg/ml) of *V. officinalis*, which yields better FCR but negatively affected the blood and serum profile severely, the exact reason of which is unclear but may be possible due to overdose.

The derivatives of *V. officinalis* showed anticancer activities with LC_{50} < 20 mg/ml in a human cell line (Encalada et al., 2015). It also assists in the regulation of human lactation and menstrual cycle (Hernandez et al., 2000), curing rheumatism and joint pain (Li et al., 2003). In comparison marketed ointment piroxicam, *V. officinalis* is effective against edema (Calvo, 2006). The oil derivatives of *V. officinalis* have an apoptotic effect in chronic lymphocytic leukemia (Martino et al., 2011). The methanolic extract of *V. officinalis* has shown 30% antifungal activity against *Rhizopus stolonifer* and *Penicillium expansum* (Casanova et al., 2008), while the culicidal activity with LC_{50} of 38 ppm (Pavela, 2009). The methanolic crude extract of *V. officinalis* has antibacterial activity against *Staphylococcus aureus* (Sahile et al., 2015). The purpose of selecting *P. glabrum* against avian coccidiosis is that it exhibits some qualities and different activities have been done with it against different diseases and pathogens as elaborated above, in addition, the member of the same genus "*Polygonum aviculare*" was found effective against coccidiosis (Youn and Noh, 2001).

The level of AST in chicks rises with coccidiosis infection (El-Maksoud et al., 2014), same is the case with the current experiment which show elevation of AST in the infected groups earlier but later on, the healing potential of *V. officinalis* and *P. glabrum* decreased its level. In the current study, the results of increasing the level of protein with decreased ALT and AST profile in the recovered groups were comparable with the findings of Cowieson (2020). The thiamine receptors help in carbohydrate synthesis which enhances *Eimeria* oocysts sporulation, the synthetic drug amprolium as its antagonist blocks the thiamine receptor and inhibit sporulation process (Ali and Abdelhalim, 2020). Prinzo and collegues (1999) reported the flavonoids as antagonist to thiamine. Considering the blockage of thiamine receptors, the flavonoids may block the thiamine receptor like amprolium due to its antag-

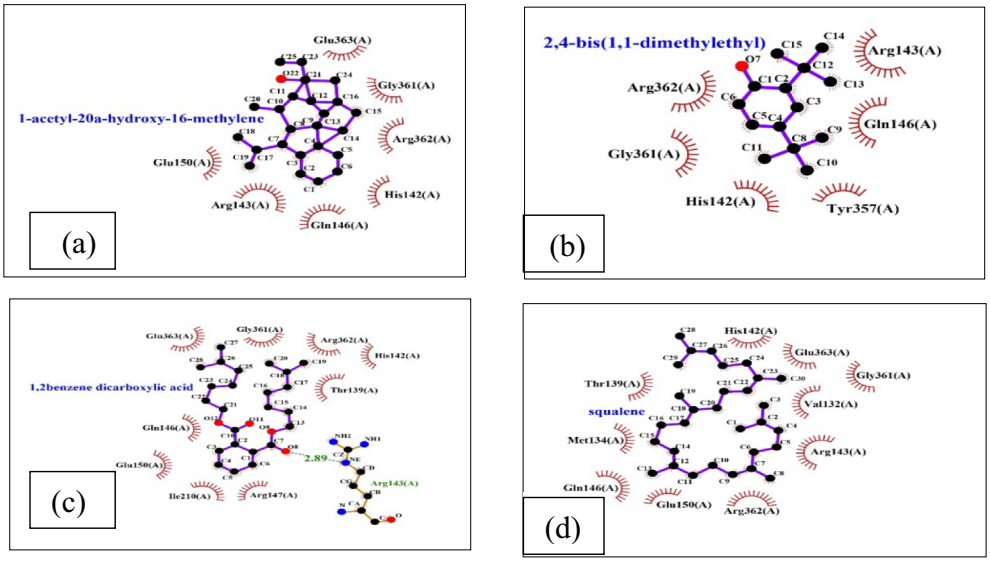

Fig. 5. 2D representation of protein-ligands complex after LigPlot + analysis (a) Strychane, 1-acetyl-20a-hydroxy-16-methylene (b) Phenol, 2,4-bis(1,1-dimethylethyl) (c) 1,2benzene dicarboxylic acid, diisooctyl ester (d) Squalene. Hydrophobic interactions are represented by red spiky arcs, while hydrogen bonds are indicated by green dashed lines along with calculated bond length.

onistic behavior and act as anticoccidial agent. Further, the effectiveness of the studied herbal extracts was supported by the results of different hematological, biochemical parameters, and histomorphology. Additionally, the symptoms like oocyst abundance or bloody diarrhea, the common indicators of coccidiosis, are also used to evaluate the potency of *V. officinalis* and *P. glabrum* that either any change in those parameters occurs with the treatment of the respective concentrations of the studied herbs. The phyto-components of methanolic crude extract of *V. officinalis* exhibits some biological activities which enhance its importance, some of the properties are sited in this work. The anti-microbial activity organic compounds like Tetradecane (Idris et al., 2019), Hexadecane (Padma et al., 2019), 6-Octadecenoic acid methyl ester (Adegoke et al., 2019), Linoleic acid ethyl ester (Huang et al., 2010), Hexacosane (Banakar and Jayaraj, 2018) were reported. The decane (Idris et al., 2019), and phenol, 2,4-bis(1,1-dimethylethyl)- (Devi et al., 2021) were found active against fungus. Dodecane (Padma et al., 2019), Eicosane (Idris et al., 2019), and Heptacosane (Khatua et al., 2016) exhibits antibacterial property. The hexadecane (Padma et al., 2019), hexadecanoic acid, methyl ester (Sermakkani and Thangapandian, 2012), and squalene, 1-Monolinoleoyl glycerol trimethylsilyl ether (Banakar and Jayaraj, 2018) were reported as antioxidant agents. The anti-cancerous activity of Squalene, 1-Monolinoleoyl glycerol trimethylsilyl ether was also noticed by Banakar and Jayaraj in 2018. Ecosane has cytotoxic activity (Idris et al., 2019), whereas tetratetracontane is cyto-protective in nature (Amudha et al., 2018). The compounds like octacosane (Rajkumar and Jebanesan, 2004), hexadecanoic acid, methyl ester (Sermakkani and Thangapandian, 2012) have best insecticidal activity. The Sulfurous acid, cyclohexyl methyl octadecyl ester has synergistic behavior by acting as catalyst or enhancing the rate of reaction (Rukaiyat et al., 2015). The octadecane is used for the treatment of phobic disorders (Kumaresan et al., 2015). 1,2 benzene dicarboxylic acid, diisooctyl ester is used in the synthesis of various perfumes and cosmatics (Hema et al., 2011).

5. Conclusion

In the current study, it is concluded that the aqueous and methanolic crude extracts of *V. officinalis* and *P. glabrum* showed significant activity against *E. tenella* sporulating oocyst in terms of low IC_{50} as compared to *M. arvensis* (*in-vitro*). Similarly, the groups treated with methanolic crude extracts of *V. officinalis* (*in-vivo*) results in enough weight gain and good FCR with recovered hematological and biochemical profile, bearing no negative impact. The molecular docking of phyto-components of *V. officinalis* suggests: 1,2benzene dicarboxylic acid, diisooctyl ester exhibits anticoccidial potential. More extensive investigation of V. officinalis should be carried out, especially bio-guided fractionation to find the most effective fraction against the avian coccidiosis and further the chemical characterization of compound structures.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contribution

SAAS and NAQ conceived and designed theoretical framework. SAAS conducted experiments; AS help in data entry evaluation. AZ assisted in molecular modeling. MZQ and SSA proof read the publication and wrote the manuscript. All the authors read and approved the manuscript.

Data Availability

The dataset of the current study are available from the corresponding author on reasonable request.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2023.103646.

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