Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2020 17(4): 2760-2769. **OPEN ACCESS**

Assessment of *in vitro* anthelmintic activity of crude extracts of *Dysphania ambrosioides* against *Haemonchus contortus* a sheep pathogenic parasite

Muhammad Ajaib*[1], Saleha Farooq[2], Samia Abid[1], Faiza Shafi[1], Muhammad Fiaz Qamar[3] and Muhammad Tariq Zahid[4]

[1] Department of Botany, Mirpur University of Science & Technology (MUST), Mirpur-10250 (AJK), **Pakistan**
[2]Department of Botany, Government College University Lahore, **Pakistan**
[3]College of Veterinary & Animal Sciences, 12-Km Chiniot Road, Jhang. **Pakistan**
[4]Department of Zoology Government College University Lahore, **Pakistan**

***Correspondence:** majaibchaudhry@yahoo.com **Received 14-07-2020, Revised: 01-11-2020, Accepted: 120-11-2020 e-Published: 20-11-2020**

The petroleum ether, methanol, chloroform and aqueous extract of *Dysphania ambrosioides* (L.) Mosyakin & Clemants obtained by maceration technique. Before starting anthelmintic activity, phytochemicals was assessed for crude plant extracts and it was revealed that anthraquinones, reducing sugar, tannins, saponins, flavonoids, alkaloids and cardiac glycosides are present while all extracts showed positive results for tarpenoids. *In vitro* anthelmintic activity of the selected plants was determined on intestinal parasite of sheep/goat, which resembles with the intestinal worms of human being, i.e. *Haemonchus contortus* (Rudolphi) Cobb and the activity was carried out by noting the time of paralysis (P) and death (D) of worm at specific concentration. Four concentrations were made of four solvents. Chloroform bark extract took minimum time for paralysis and death of worms, i.e. 12±0.8 and 17±0.5 at 100mg/mL concentration whereas maximum time taken by also chloroform extract for paralysis with a value 99±0.5 and 142±1.1 of petroleum ether extract of fruit at 20mg/mL. Chloroform extract of fruit took minimum time for paralysis and death of worms, i.e. 10±1.1 and 15±1.1 at 100mg/mL concentration whereas maximum time taken by also chloroform fruit extract for paralysis of worm value was 98±1.3 and 139±1.2 in petroleum ether at 20mg/mL. Chloroform extract also exhibited minimum time duration for paralysis and death at 80mg/mL. Investigation of anthelmintic activity exhibited that fruit of *D. ambrosioides* more potent than of bark.

Keywords: *Dysphania ambrosioides* (L.) Mosyakin & Clemants, Phytochemicals, Anthelmintic Activity

INTRODUCTION

Phytochemical compounds extracted from plants play a crucial role in medicine and they have been a basis of medicine for thousands of years (Ajaib et al. 2020). The demand of medicinal plants is rising day by day due to their increased prominence and distinction; conversationalist recommended that wild medicinal plants should be cultivated. Plenty of medicinal plants in addition to their purified constituents had been revealed valuable therapeutic and remedial potential (Ajaib et al. 2017a).

Plants have been used as a remedy for many diseases since a long time, as they have therapeutically strong constituents (Ishtiaq et al. 2018). Plant metabolites have been appreciably prescribed to cure various ailments. Demand of medicinal plants is increasing due to occurrence of remedial natural products in plants having no or

minimum side effects and after establishment of their pharmacological standards these natural products as a crude drug can be integrated in herbal pharmacopoeia (Siddiqui et al. 2017). Since ancient times, plants frequently use for the cure of infectious diseases because of its great potential in the research of antimicrobial constituents (Ajaib et al. 2017b). Plants have capability of synthesizing the secondary metabolites against insects, animals and microorganism which is adopted due to defensive mechanism of plants. Therefore, as results of million years of adaptation of plants, facilitating a valuable source of new molecules for researching antibacterial and antifungal activities, plants are regarded as organic synthesis laboratory (Ajaib et al. 2016).

The selected plant, *Dysphania ambrosioides* belongs to Chenopodiaceae family and it is consisted of 103 genera and about 1300 species worldwide where as in Pakistan its contribution is 35 genera, and 106 species. These genera are largely distributed in desert, semi-desert and along sea-shores (Ajaib et al. 2016). *D. ambrosioides* is strongly aromatic, annual to short lived perennial with erect ascending stem. Leaves lanceolate to elliptical. Seeds mostly horizontal, sometimes oblique, brown (Fig. 1).

Figure 1.1: *D. ambrosioides* in natural habitat

It is probably originated from Tropical America. Earlier cultivated as medicinal plant and introduced in tropical and subtropical areas of the world, where it is commonly naturalized (Freitag et al., 2001). Infusions of *D. amrosioides* have been used, in center America as a popular medication against intestinal worm infections, as vermifuge. In India, leaves are helpful in the treatment of influenza, pneumonia, and typhoid and also used as vermicide (Brahim et al. 2015).

This plant is widely and traditionally used as anti-inflammatory and anti-parasitic; moreover, antibiotics made from this plant are also use, which efficiency has been scientifically proven. The essential oil which is extracted from *D. ambrosioides* L. inhibit the growth of yeast species, dermatophytes and other filamentous fungi and the hexane extract of *D. ambrosioides* is known to inhibit the growth of filamentous fungi (Sousa et al. 2012).

MATERIALS AND METHODS

Plant material

The plant specimen was collected from District Kotli, Azad Jammu & Kashmir (AJK). The plant material identified and acknowledged to the Dr. Sultan Ahmed herbarium, Department of Botany, GC University, Lahore with the valid voucher No. GC. Herb. Bot. 2944.

Test organism

The parasitic test organism employed for investigation i.e. *Haemonchus contortus*. The abosmasum of freshly slaughtered goat was achieved from local slaughterhouse. The test organisms were obtained from the dissection of abomasums. After rinsing and washing the abosum with 0.9% NaCl solution to abandon maximum griminess. The organism was identified and authenticated from Department of Zoology, GC University, Lahore.

Methodology implemented

Plant specimen primarily distinguished into bark and fruit; uniformly spread on dishes for steady drying and placed to aridity in shadow at room temperature under optimum environmental conditions, for 20-30 days. Ultimately, the desiccated plant material was crushed and subjected for maceration.

Extraction of plant material

The technique which is considered for maceration was static- state maceration. The maceration planned from non-polar to polar solvent and solvents were applied with their polarity gradients. Quantity of the precisely crushed plant specimens were subjected to impassable glass urn and were consistently shove in the solvent. Sequentially, extracts were

obtained from the solvents i.e. petroleum ether (P.E), methanol (MeOH), chloroform (Chl.) and distilled water (Aq.). Firstly, compacted plant powdered rinsed in petroleum ether (500mL) for 8 days.

Subsequently, after the accomplishment of the time, the constituents which are present in glass bottle were alienated in the course of filtration by means of filter paper. The extract of plant material was preserved in glass vial; solid filtrate was dried out and similar progression was tried for the methanol and chloroform macerate with the similar time period and then for further processing it is referred to filtration. Finally, the solid remains which are in dried form was soaked in 500mL distilled water and then referred to filtration after interval of 8 days. Eventually, extracts of petroleum ether, methanol, chloroform and distilled water were concentrated and dehydrated by means of rotary evaporator. The temperature should be upto 40°C to evade the crumbling of thermolabile ingredients and lyophilizer was used for aqueous extract. The concentrated extract which is obtained, stored at 20°C.

The physical investigation of plant extracts take account of its colour, appearance and texture and percentage (%) yield before further scrutiny was conducted. The percentage (%) yield was calculating by using the formula which is given below:

$$\text{Percentage}(\%)\ \text{Extraction yield} = \frac{\text{Wt. of plant extract}}{\text{Wt. of initial plant sample}} \times 100$$

Phytochemical tests

Phytochemical tests of plant extracts were executed qualitatively.

Qualitative test

Qualitative analysis of plant extracts were carried out for the estimation of presence or absence of alkaloids, phenols, flavonoids, tannins, saponins, terpenoids and cardiac glycosides.

Grounding of 2 mg/mL plant extract

Weigh 0.02g of plant extract and subjected to glass vial and raise the volume upto 10mL with solvent in which extract was prepared petroleum ether, methanol, chloroform and distilled water.

a. Test for alkaloids

Mayer's test

The procedure of Sethi (2003) was followed for this experiment. Pipette out 1mL of 2mg/mL plant extract added in test tube afterward add 0.2mL of 2M HCl and 1mL of freshly prepared Mayer's reagent.

Wagner's test

Wagner (1993) methodology was implemented for alkaloid investigation. 1mL plant extract was added in reaction tube and 0.2mL 2M HCl and 1mL of Wagner's solution was also added.

b. Test for reducing sugars

Fehling's test

2mL of Fehling's solution, 3 droplets of plant extract were added in the test tube and subjected to water bath for few minutes at 60°C.

C. Test for terpenoids

Salkowski test

This test named following the German biochemist Ernst Leopold Salkowski was taken into deliberation of adhering Harborne (1973). Weigh 0.5g of plant extract and 2mL chloroform was transferred to reaction tube and carefully add 30mL of concentrated H_2SO_4 to create a stratum.

d. Test for tannins

Ferric chloride test

Evans (2009) put forward the procedure for the confirmation of tannins. Weigh 0.25g of plant extract and add 5mL of distilled water in test tube, boiled, filtered and 3 drops of 0.1% ferric chloride ($FeCl_3$) was added in filtrate.

e. Test for saponins

Frothing test

Akinjogunla et al. (2010) referred a method for the estimation of saponins. 0.5g of plant extract measured accurately was added in 5mL of autoclave water in test tube. Water bath is used for constant heating and shudder dynamically and wait for the stable froth**.**

f. Test for flavonoids

Sodium hydroxide test

Bello et al. (2011) referred a method for sodium hydroxide test. Weigh accurately 5mg of plant macerate and added 2mL distilled water and heat it. Consequently, 2mL of 10% sodium hydroxide was also added in test tube.

Ferric chloride test

Mace (1963) established methodology for $FeCl_3$. Accurately measured 5mg of plant was mixed with 2mL methanol which followed by some droplets of 10% ferric chloride solution.

g. Test for cardiac glycosides

Keller-Killiani test

Keller-Killiani test was devised by Onwukaeme *et al.* (2007). Pipette out 2mL of glacial acetic acid in test tube, also added one droplet of ferric chloride and 0.5mL of 10mg/mL plant extract subjected to test tube. In the end, addition of 1mL of concentrated sulphuric acid was conceded out by the face of reaction tube.

h. Test for anthraquinones

Borntrager's test

After Evan (2009), Borntrager's test was commenced for the estimation of anthraquinones. Precisely measured the 0.25g of the plant extract followed by 5mL of dilute H_2SO_4 and then boiled for 2 minutes; filtered the mixture when it is hot. After filtration, addition of 2.5mL of chloroform was carried out in test tube; dynamic stirring caused appearance of distinct organic layer. In the end, layer was separated and subjected to another test tube followed by 0.5mL ammonium hydroxide.

Anthelmintic Activity

Following method was considered for the evaluation of anthelmintic potential of *Chenopodium ambrosioides* L.

Preparation of 0.9% saline *solution*

Through serial dilution method, four concentrations (20, 50, 80, 100mg/mL) of plant extract were prepared and accurately calculated the amount of solute to the glass vials. The final volume raised upto 20mL with the particular solvent in which the plant matter was macerated.

Procedure

Ajaiyeoba et al. (2008) established the methodology for the anthelmintic potential of plants macerates at the different concentrations (20, 50, 80 and 100mg/mL). In each of first two petri- plates, 10mL of Piperazine citrate for standard and 0.9% NaCl for control and other plates plant macerates were distributed followed by the addition of same-sized worms. All plates were located for about 4 hours at room temperature for the testing session

RESULTS AND DISCUSSION

The method used for the extraction of the phytochemical compound was measured to be a crucial factor because different parameters were considered in this regard i.e. commercial viability, extraction yield and its quality. The highest yield obtained from bark of *D. ambrosioides* i.e. 15.45%. The % yield of fruit of *D. ambrosioides* was 15.1%. The lowest yield depicted by fruit of *D. ambrosioides* was 5.41% (Fig. 2).The difference in percentage extraction yield is because of two reasons. First of all, the % extraction yield increases progressively alongside with the increase in the solvents' polarity, i.e. the % extraction yield has directly proportional relation to the solvents' polarity index. Secondly, special compounds at hand in the plant matter were soluble in their distinctive solvents and the division of the components was potholed in the various parts of the plant as described by Ajaib et al. (2014) in evaluation of biological activities of *Firmiana simplex.*

Phytochemical screening was executed qualitatively for the estimation of compounds in plant material. Several tests were performed to investigate the presence or absence of chemical compounds i.e. flavonoids, terpenoids, tannins, alkaloids, anthraquinones, saponins and cardiac glycosides. The macerates that have compounds in it, categorize in three types weak, moderate and strong quantity of chemical compounds in respective plant matter (Table-1). Tannins present in all extracts except aqueous fruit extract whereas all extracts showed that terpenoids is present in them (Fig. 3).

Anthelmintic activity of *D. ambrosioides* was carried out by noting the time of paralysis (P) and death (D) of worm at specific concentration (Fig. 4). Chloroform extract took minimum time for paralysis and death of worms i.e. 12±0.8 and 17±0.5 at 100mg/mL concentration whereas maximum time taken by also chloroform extract for paralysis and death of worms i.e. 99±0.5 and 115±0.3 at 20mg/mL (Table 2) somewhat similar results were also reported by Ajaib et al. (2019) during evaluation of anthelmintic activity of crude

extracts of *Salsola imbricata*.

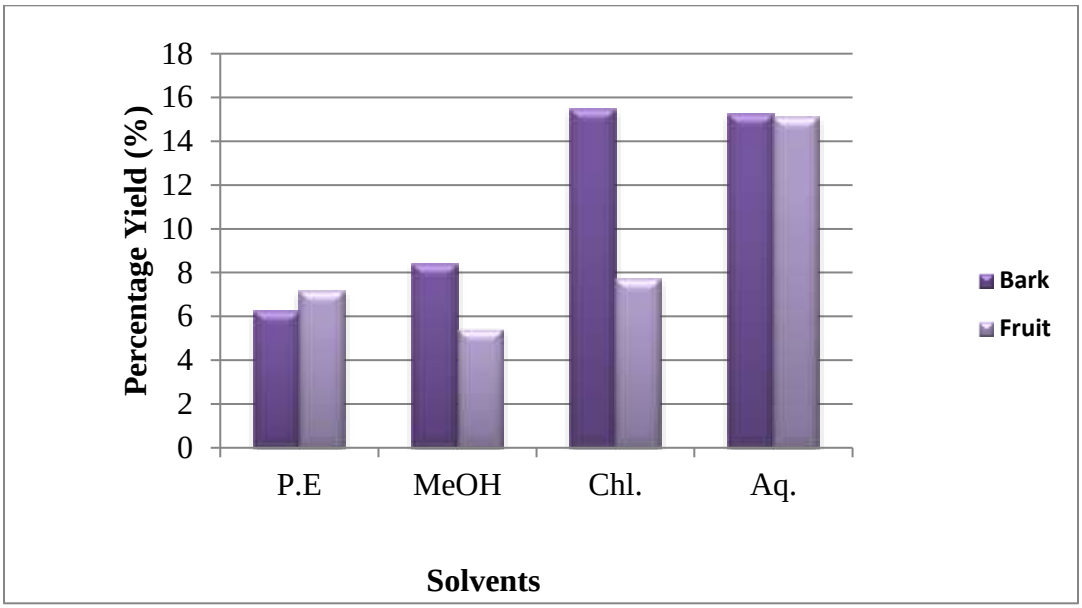

Figure 2: Extraction yield (%) of different parts of *D. ambrosioides*

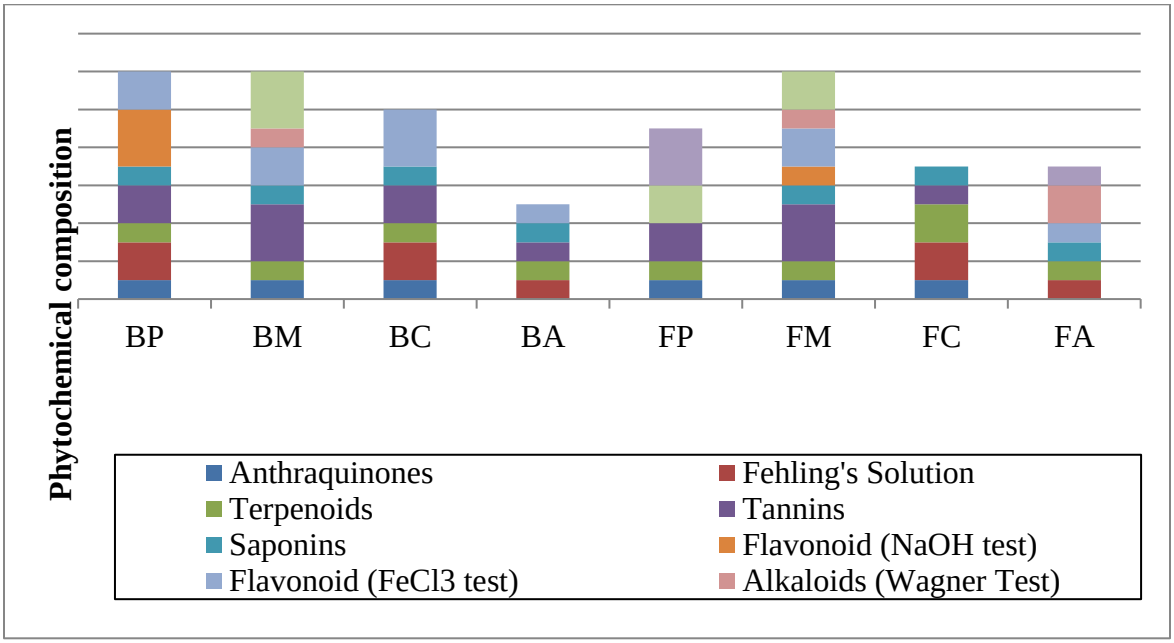

Figure 3: Phytochemical composition of crude extracts of bark and fruit of *D. ambrosioides* L. in different solvents

Table 1: Phytochemical tests of bark and fruit of *D. ambrosioides*

Plant Parts	Solvents	Presence/ absence of phtyochemical constituents									
		Anthraquinones	Fehling's Solution	Terpenoids	Tannins	Saponins	Flavonoid (NaOH test)	Flavonoid (FeCl3 test)	Alkaloids (Wagner Test)	Alkaloids (Mayer's Test)	Cardiac glycosides
Bark	Petroleum Ether	+	++	-	+++	+	++	+++	+	-	+++
	Methanol	+	-	+	+	+++	++	-	++	++	-
	Chloroform	-	+	+++	+	++	+	-	-	-	++
	Aqueous	-	+	-	-	+	+	++	++	+	-
Fruit	Petroleum Ether	-	-	+	+++	-	+	-	-	++	+++
	Methanol	+++	-	++	+	+++	+	+	++	+++	-
	Chloroform	-	++	+++	+	++	+	-	-	-	-
	Aqueous	+	+	-	-	+	-	+	++	+	+

*Phytochemical detection key: − = Absent, + = Present in minimal quantity, ++ = Present in medium quantity, +++ = Present in appreciable quantity.

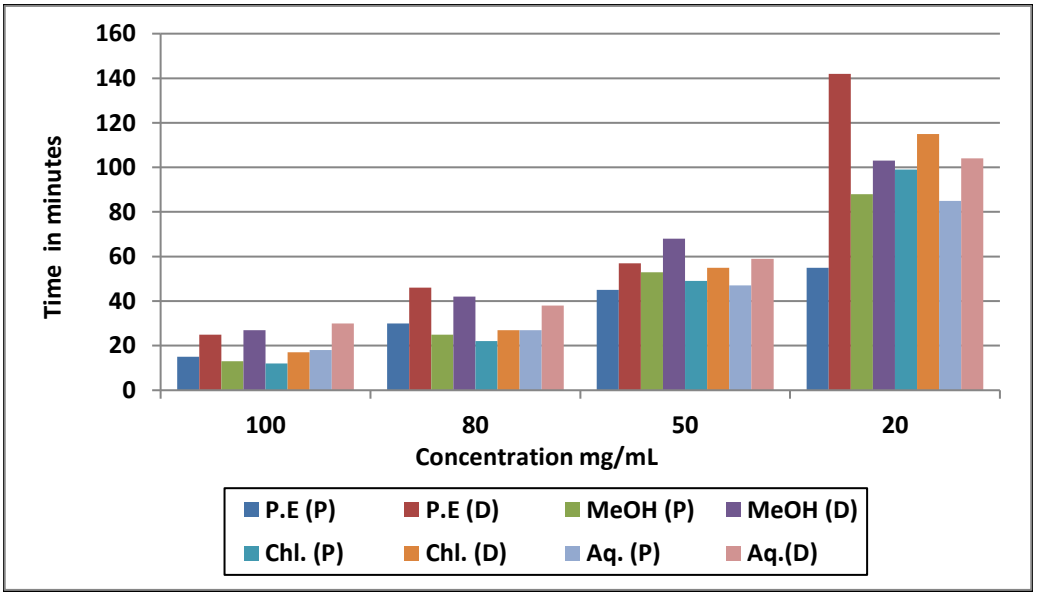

Figure 4: Anthelmintic activity of *D. ambrosioides* bark extract

Table 2: Anthelmintic activity of *D. ambrosioides* bark extract

Solvents	Conc. (mg/mL)	Time taken for Paralysis (P) in minutes	Time taken for Death (D) in minutes
Petroleum ether	100	15±0.5	25±0.5
	80	30±1.2	46±0.5
	50	45±0.1	57±0.3
	20	55±0.5	142±1.1
Methanol	100	13±0.3	27±1.5
	80	25±0.7	42±0.5
	50	53±0.6	68±1.5
	20	88±0.5	103±0.4
Chloroform	100	12±0.8	17±0.5
	80	22±1.3	27±0.1
	50	49±0.1	55±0.4
	20	99±0.5	115±0.3
Aqueous	100	18±0.5	30±1.1
	80	27±0.4	38±1.0
	50	47±1.1	59±0.6
	20	85±1.5	101±0.3

Table 3: Anthelmintic activity of *D. ambrosioides* fruit extract

Solvents	Conc. (mg/mL)	Time taken for Paralysis (P) in minutes	Time taken for Death (D) in minutes
Petroleum ether	100	16±0.5	27±1.1
	80	29±1.9	48±0.4
	50	47±1.1	55±1.5
	20	58±0.8	139±1.2
Methanol	100	12±0.6	25±0.6
	80	27±0.3	45±0.7
	50	55±0.4	67±0.9
	20	89±0.7	105±0.1
Chloroform	100	10±1.1	15±1.1
	80	21±0.5	28±1.1
	50	47±0.3	58±0.5
	20	98±1.3	113±1.5
Aqueous	100	15±0.5	28±0.4
	80	26±1.6	35±0.1
	50	45±0.2	60±0.2
	20	83±1.5	105±0.5

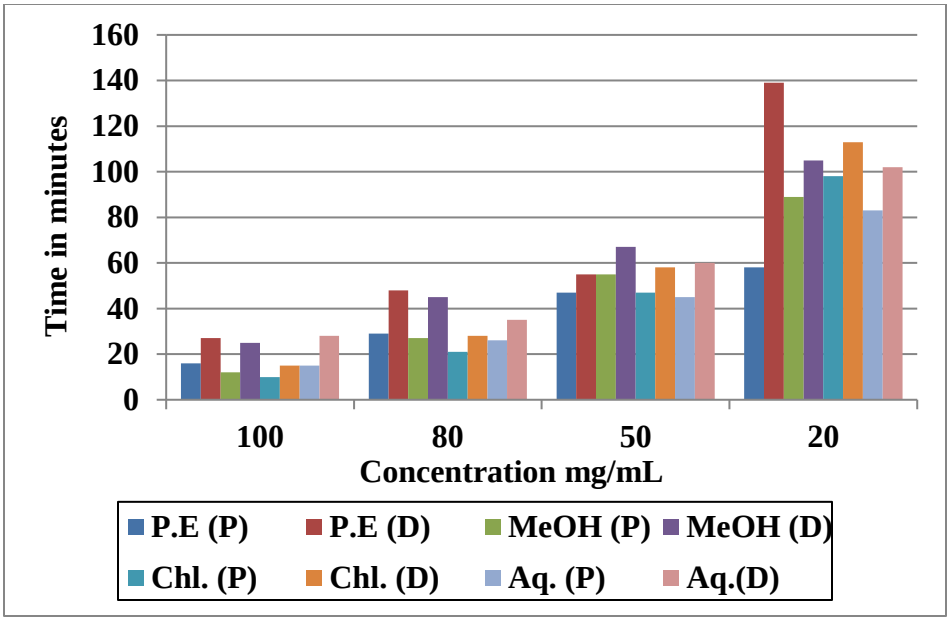

Figure 5: Anthelmintic activity of *D. ambrosioides* fruit extract

Chloroform extract took minimum time for paralysis and death of worms, i.e. 12±0.8 and 17±0.5 at 100mg/mL concentration as described by Ajaib et al. (2017b) in analysis of anthelmintic activities of *Andrachne cordifolia* and *Flueggea virosa* of family Euphorbiaceae whereas maximum time taken by also chloroform extract for paralysis and death of worms i.e. 99±0.5 and 115±0.3 at 20mg/mL (Table 3). Chloroform extract also exhibited minimum time duration for paralysis and death at 80mg/mL. Aqueous extract exhibited minimum time for paralysis i.e. 45±0.2 at 50mg/mL (Fig.5).

Chloroform extract of selected plants displayed minimum time for paralysis and death. The results obtained from the anthelmintic activity of *Chenopodium ambrosioides* L. revealed that paralysis depends upon dose of macerate, increase in dose, and results loss of response to external stimulus which eventually leads to death. These findings were same as discussed earlier by Partap et al. (2012) in evaluation of anthelmintic activity of *Luffa species*.

CONCLUSION

The phytochemical screening of extracts showed occurrence of anthraquinones, tarpenoids, tannins, saponins, reducing sugar, flavonoids, alkaloids and cardiac glycosides. Most extracts showed good potential of tarpenoids and tannins as compared to other compounds. Investigation of anthelmintic activity displayed that fruit of plant had more potential than their bark and chloroform extract show maximum potential as compared to others. The intention of this study to evaluate the potential of plant *Dysphania ambrosioides* (L.) Mosyakin & Clemants (*Chenopodium ambrosioides* L.). The results exhibited *D. ambrosioides* that had showed great potential and further studies might reveal some new bioactive compounds that would make contribution to the ethnopharmacology..

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

AUTHOR CONTRIBUTIONS

MA designed and SFperformleed the experiments and both also wrote the manuscript. SA, FS, MFQ and KHB help in experiments and reviewed the manuscript. All authors read and approved the final version.

REFERENCES

Ajaib M, Ashraf Z and Siddiqui MF, 2017a. *Cocculus laurifolius*: A rich antimicrobial, antioxidant and phytochemical source. Pak. J. Bot. 49(1): 337-344.

Ajaib M, Farooq S, Khan KM, Perveen S and Shah S, 2019. Phytochemical Analysis and Anthelmintic Activity of *Salsola imbricata*. J Chem Soc Pak. 41(1): 198-202.

Ajaib M, Hussain T, Farooq S and Ashiq M, 2016. Analysis of Antimicrobial and Antioxidant Activities of *Chenopodium ambrosioides*. Journal of Chemistry 1: 1-11.

Ajaib M, M Ishtiaq, Shafi F, Bhatti KH and Zahid MT, 2020. Antimicrobial and antioxidant analysis of *Strobilanthes glutinous*: an unexplored medicinal plant. Bioscience Research 17(3): 1521-1534.

Ajaib M, Mehak T, Fareed S, Perveen S and Shah S, 2017b. Antimicrobial and Antioxidant activity of *Citharexylum Spinosum.* J. Chem. Soc. Pak. 39(2): 254-260.

Ajaib M, Wahla SQ, Khan KM, Perveen S and Shah S, 2014. *Firmiana simplex:* A Potential source of antimicrobials. J. Chem. Soc. Pak. 36: 744-752.

Ajaib M, Wahla SQ, Wahla UG, Khan KM, Perveen S and Shah S, 2018. Phytochemical Screening and Anthelmintic Activity of *Flueggea virosa.* J. Chem. Soc. Pak. 40(4): 702-706.

Ajaib M, Wahla SQ, Wahla UG, Perveen S and Shah S, 2017. Phytochemical Screening and Anthelmintic Activities of *Andrachne cordifolia.* J. Chem. Soc. Pak. 39(3): 429-433.

Akinjogunla OJ, Yah SC, Eghafona NO and Ogbemudia FO, 2010. Antibacterial activity of leave extracts of *Nymphaea lotus* (*Nymphaeaceae*) on Methicillin Resistant *Staphlyococcus ayreus* (*MRSA*) and Vancomycin Resistant *Staphylococcus aureus* (*VRSA*) Isolated from clinical samples. Annals of Biological Research 1(2): 174-184.

Ayoola GA, Adesegun AA, Obaweya K, Atangbayila TO, 2008. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestren Nigeria. Tropical Journal of Pharmaceutical Research 7: 1019-1024.

Bello IA, Ndukwe GI, Audu OT and Habila JD, 2011. A bioactive flavonoid from *Pavetta crassipes* K. Bioorg. Med.Chem. Lett. 1(14): 1-5.

Brahim MAS, Fadli M, Hassani L, Boulay B, Markouk M, Bekkouche K, Abbad A, Ali MA and Larhsini M, 2015*. Chenopodium ambrosioides* var. *ambrosioides* used in Moroccan traditional medicine can enhance the antimicrobial activity of conventional antibiotics. Industrial crops and products 71: 37-43.

Evans WC, 2009. Trease and Evans pharmacognosy. 15thEdition WB Saunders Ltd. London. Pp: 137-159.

Freitag, H., Hedge IC, Jafri SMH, Kothe-Heinrich G, Omer S and Uotila P, 2001. *Flora of Pakistan*. Chenopodiaceae. No. 204. (Ali, S. I. and Qaiser, M. eds.) Department of Botany, University of Karachi.

Harborne JB, 1973. *Phytochemical Method*, London, Chapman and Hall, Ltd. Pp: 49-188.

Ishtiaq S, Hanif U, Ajaib M, Shaheen S, Afridi MSK and Siddiqui MF, 2018. Pharamcognostical and Physicochemical Characterization of *Amaranthus Graecizans* Subsp. *Silvestris*: An Anatomical Perspective. Pak. J. Bot. 50(1): 307-312.

Mace ME, 1963. Histochemical localization of phenols in healthy and diseased tomato roots. Phytopathology 16: 915-925.

Onwukaeme DN, Ikuegbvweha TB, Asonye CC, 2007. Evaluation of phytochemical constituents, antibacterial activities and effect of exudate of *Phycanthus angolensis* Weld Warb (Myristicaceae) on corneal ulcer in rabbits. Tropical Journal of Pharmaceutical Research 6(2): 725-730.

Partap S, Kumar S, Kumar A, Sharma NK, Jha KK, 2012. In-Vitro Anthelmintic Activity of Luffa Cylindrica Leaves in Indian Adult Earthworm. Journal of Pharmacognosy and Phytochemistry. 1(2): 30.

Sethi A, 2003. Lab experiments in organic chemistry, New Age International (P) Limited, Publisher, ISBN 81-224-1491-5.

Siddiqui SZ, Saleem H, Abbasi MA, Aziz-ur-Rehman and Ajaib M, 2017. *Lonicera quinqueloculari*s: A rich source of antioxidant for protection against chronic diseases. Pak. J. Pharm. Sci. 30(2): 347-353.

Sousa ZL, de Oliveria DD, da Conceicao AO, Silva LAM, Rossi MH, Santos JDS and Andrioli JL, 2012. Biological activities of

extracts from *Chenopodium ambrosioides* Lineu and *Kielmeyera neqlecta* Saddi. Annals of clinical microbiology and antimicrobials, 11:20.

Wagner, H. 1993. Pharmazeutische Biology 5th edn. AUFI.15 BN 3-437-20 498-x. Gustav fisher Vwelag. Stuttgart, Germany.