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CHEMICAL PROFILING OF ETHYL ACTATE FRACTION OF *GALIUM TRICORNE* STOKES AND ITS PHYTOTOXIC AND ANTHELMINTIC EVALUATION

Ghallab Alotaibi[1#], Sohrab Ali Khan[2], Haroon Khan[2], Sajjad Ahmad[3*], Saba Zafar[4]

[1]Department of Pharmacology, College of Pharmacy Al-Dawadmi Campus, Shaqra University, Shaqra, KSA, Email: ghalotaibi@su.edu.sa
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy Gomal University Dera Ismail Khan KP Pakistan, Email: sohrabaliwazir@gmail.com, Email: drharoon@gu.edu.pk
[3]Cadson College of Pharmacy Affiliated with Punjab University, Lahore, Punjab, Pakistan, Email: sajad4u2000@yahoo.com
[4]Department of Biochemistry and Biotechnology, The Women University Multan, Punjab, Pakistan, Email: saba.6498@wum.edu.pk

***Corresponding Author:** Sajjad Ahmad
*Cadson College of Pharmacy, Affiliated with Punjab University, Lahore, Punjab, Pakistan, Email: Sajad4u2000@yahoo.com

#Co-corresponding Author: Ghallab Alotaibi
#Department of Pharmacology, College of Pharmacy, Al-Dawadmi Campus, Shaqra University, Shaqra, KSA, Email: ghalotaibi@su.edu.sa

Abstract

The emergence of different diseases is of great concern globally. To treat these challenging diseases effectively is based on the discovery of potent drugs. Medicinal plants are the important source for the discovery of effective, economical and safe drugs. In order to authenticate its phytotoxic and anthelmintic effects, we have screened for the first time *Galium tricorne* Stokes against radish (*Raphanus sativus* L.) seeds and variety of helminthes. Besides these biological assays different compounds have been identified in ethyl acetate fraction (Gt.Eta) and sorted out on the basis of biological potential through Gas Chromatography-Mass Spectrometry (GC-MS) technique. Crude methanolic extract (Gt.Crd)and fractions of *Galium tricorne* Stokes were tested against radish seeds and different helminthes; adult earth worms (*Pheretima posthuma*), Round worms (*Ascaridia galli)*, and tape worms (*Raillietina spiralis*). In phytotoxicity assay Chloroform (Gt.Chf) and Gt.Eta exhibited more phytotoxic effect at different concentration of 10, 100 and 1000 µg/mL as compared to other plant samples against radish seeds. The IC_{50} values for Gt.Chf and Gt.Eta against radish seeds root length inhibition and germination inhibitionwere 12.65, 21.36 µg/mL and 12.20 and 25.54 µg/mL respectively. The plant sample in concentration of 10, 20 and 40 mg/mLwasalso documented with prominent anthelmintic effects against *Pheretima posthuma*, *Ascaridia galli* and *Raillietina spiralis*.22 compounds were identified in Gt.Eta fraction through GC-MS analysis and several bioactive compounds were sorted out having biological potential.This research provided pharmacological background for the ethnomedicinal uses of *Galium tricorne*.

Keywords: Anthelmintic, Phytotoxic, *Galium tricorne*, GC-MS analysis and ethyl acetate fraction

INTROUCTION

The country Pakistan is gifted naturally with large number of medicinal plants because of diverse edaphic conditions and climate. Currently six thousand flowering plants have been reported from Pakistan out of which 400-600 are of highly medicinal potential [1]. In developing countries indigenous phyto-therapy is still used, although a large number of effective drugs have been developed [2]. In primary health care medication about 85% medications are obtained worldwide from natural sources [3]. It is anticipated to employ the biological and therapeutic effects of medicinal plants to obtained effective, safer and affordable novel natural drugs.

Galium tricorne belongs to *Rubiaceae* family. *Galium* Linnaeus is one of the largest genera within the family *Rubiaceae* and consists of approximately 650 species [4]. Species belong to *Galium* genusis used traditionally to coagulate milk because of the presence of enzyme in their chemical composition. Therefore, these plants are called as "yogurt herb" [5].

Extracts of the *Galium* species have long been used in folk medicines for various disorders like, astringent, choleretic, diuretic, gout, epilepsy and treatment of stomach diseases [6, 7]. It has been reported that *Galium tricorne* possess antibacterial, cytotoxic and anticancer effect [8].

About 02 billion peoples globally are parasitic worms carriers of different ages [9]. These parasitic worms causes malabsorption, iron deficiency anemia, malnutrition and causes small intestine obstruction which further lead to impairment intellectual, cognitive potential and physical growth in children [10,11]. It is very important to aware the community about the treatment of infected individual patient [12]. A common animal disease in developing country is helminthiasis leading to less production of milk [13].

In the developing countries novel anthelmintic drugs development is not very rapid because of less financial assistance to that of investment. Besides this, advent of anthelmintic drugs resistance by the helminthes has led to the suggestion of medicinal plants screening for their anthelmintic potential [14]. Consequently, for the treatment of parasitic infections there is need to develop novel drug, effective with minimum cost [15]. Various medicinal plants have been employed against parasitic infections in animals and human beings due to having anthelmintic compounds [16, 17].

Weeds are the most responsible and key factor in the reduction of crops yield. In Pakistan especially in Sindh and Punjab provinces about forty weeds species leads to 40% loss in wheat crops. Different chemicals are used to thwart these undesirable weeds. Though the use of these chemicals is restricted due to residual toxicity, high cost, environmental pollution and carcinogenicity [18, 19, 20]. Accordingly alternative herbicides are needed which are economical, safer and effective. Natural herbicides may be the best candidates among these alternatives.

METHODS

Collection and identification of plant

The plant was collected from Kot Sadat, Ghoriwala District Bannu, KhyberPakhtunkhwa, Pakistan in blooming season of April and May. The identification of plant was done by taxonomist Dr. Tahir Iqbal, Department of Botany, University of Science &Technology, Bannu KP Pakistan. Its specimen was deposited in the herbarium, Departmentof Botany, University of Science & Technology, Bannu Khyber Pakhtunkhwa Pakistan with voucher specimen no. SA/2018/Gt/01for future reference.

Extraction and fractionation

The plant material was washed with water and shaded dried for 15 days. After drying, it was pulverized weighing approximately 7 kg. This quantity of plant powder was soaked in 32 liters of methanol (80%) and placed for soaking up-to 12 days. Filtration process was carried out to obtain filtrate. By using rotary evaporator, the filtrate was subjected under reduced pressure at 40 ºC. The concentrated form of plant extract was converted into solid Gt.Crd heating on water bath at 37 ºC [21].

For the fractionation of Gt.Crd, solvent-solvent extraction process was used on thebasis of solvent polarity starting with less polar solvent *n*-hexane towards high polar solvent water. The crude methanolic extract of the plant weighing approximately 475 gm was added to 500 mL of distilled water, shaked well to obtain suspension of the plant crude extract. Later on 500 mL of *n*-hexane was added in separating funnel to already prepared suspension. This mixture was shaken again vigorously and kept for some time undisturbed until two distinct layers are formed. Out of these two layers, the upper layer with low density was *n*-hexane and the lower layer was aqueous. This procedure was repeated again and again until the soluble portion of *n*-hexane of the plant extract was collected carefully. The *n*-hexane (Gt.Hex) obtained was further concentrated with rotaryevaporator. Then on the basis of polarity same process was repeated for chloroform fraction (Gt.Chf), followed by ethyl acetate fraction (Gt.Eta), and *n*-butanol fraction (Gt.Bta). Water/aqueous fraction (Gt.Aqu) was obtained at the end of the fractionation process [21].

Phytotoxicity assay

Phytotoxicity assay was performed on radish (*Raphanus sativus* L., *Cruciferae*) seeds by using Gt.Crd and various solvent fractions [22]. In this assay, two parameters of the radish seed were observed. These two parameters were root lengthand germination of seeds. Plant samples weighing 20 mg were mixed with 2 mL methanol to make stock solutions. From this solution, volume of 5, 50 and 500 µL were transferred into each petri plate to obtain different concentrations of 10, 100 and 1000 µg/mL. Sterilized filter paper (Whatman #1) was used in each petri plate and the solvent was evaporated. 5 mL of water was added to every petri plate, when the solvent was evaporated completely. The petri plate with distilled water and filter paper only acted as control. Paraquat was used as positive control. Radish seeds 20 in numbers already sterilized with 0.1% mercuric chloride were added to each petri plate. Seed germination and root length inhibition were measured after fifth day interval. The whole process was repeated in triplicate. The data was recorded as Mean ± SEM. By using the following formula number of % inhibition of root length and germination of seeds is determined.

$$\% \text{ root length inhibition} = \frac{\text{Length of root in test sample}}{\text{Length of root in control}} \times 100$$

Anthelmintic Assay

For anthelmintic assay adult earth worms (*Pheretima posthuma*), Round worms (*Ascaridia galli),* and tape worms (*Raillietina spiralis*) were used to check out the potentialof crude extract and fractions of *Galium tricorne* by using method previously described [23]. *Pheretima posthuma* were obtained from marshy water, tape wormswere collected and obtained from intestines of *Gallus spadiceus* (fowl) belonged to family *Phasianidae*. The infested intestines were further washed with normal saline solution to remove the fecal matter if any. After washing of the intestines, they were dissected to remove the helminthes and kept in normal saline solution. The average size of earth worm, round worm and tapeworm were 7-10 cm, 4-6 cm and 5-8 respectively. In this assay, adult earthworm *Pheretima posthuma* were used due to resemblance with human intestinal roundworm parasites called *Ascaris lumbricoids* in anatomical and physiological properties[24].

The helminth *Ascaridia galli* specie was suitable model for evaluating the anthelmintic activity as earlier anthelmintic drug was advocated [25,26]. The plant fractions and crude methanolic extract were dissolved in distilled water to make10, 20 and 40 mg/mL concentration. Worms like, *Pheretima posthuma, Ascaridia galli* and *Raillietina spiralis* of approximately same size and same type were placed in each 9 cm petri dish with 20 mL of test solution of Gt.Crd extract and solvent fractions. Reference standard Albendazole (10 mg/mL) was used and similarlysaline water was used as negative control [27, 28]. This protocol was used for all the three different type of worms. Fresh solutions of all the plant sample and standard drug solution were prepared before starting the assay. Paralysis of the worms were noted, when no movement could be observed except the worms were shaken vigorously. Death of the worms were confirmed showing no movement after vigorous shaking and by dipping in hot water. The data was expressed as a mean ± SEM of six worms in each group.

Compounds identification by using GC-MS technique

GC-MS analysis of Gt.Eta extract was evaluated for the identification of compound by using an Agilent USB-393752 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a HHP-5MS 5% phenylmethylsiloxane capillary column (30 m × 0.25 mm × 0.25 μm film thickness; Restek, Bellefonte, PA). It was equipped with an Agilent HP-5973 mass selective detector in the electron impact mode (Ionization energy: 70 eV). This analysis was checked out by using Elmer Clarus 500 Software Gas Chromatography fitted with capillary column Elite-5MS (5% Phenyl 95% dimethylpolysiloxane). The temperature of oven was set from 200 ºC to 150 ºC at the rate of 4 ºC per minute. The temperature was held for 5 minutes. The inlet and interface temperature were maintained at 250-280 ºC. The carrier gas helium was used at constantrate of 1.0 mL/minute. A volume of 1.0 µL of sample was injected. Energy of 70 eV was used in electron impact mass spectroscopy. Temperature of ions source and quadruple were kept at 230 to 150 ºC. The compounds were identified by using NIST library. Various compounds were identified in the plant sample. Furthermore, spectral data was used for the identification of compounds from the Wiley and NIST libraries. For the confirmation, fragmentation pattern of the mass spectra was used with data published in the literature [29, 30].

Statistical analysis

By using Graphpad prism software the analysis of results were obtained. In this case ANOVA followed by Bonferroni post test was employed for the comparison of various groups and control. Similarly, for the determination of IC_{50} values SPSS softwareand Excel sheet were used. Standard error mean (SEM) was also calculated at 95% confidence intervals.

RESULTS

Gt.Crd and all the fractions derived from the extract of *Galium tricorne* have shown phytotoxicity on radish seeds by inhibiting its germination and root length. The crude methanolic extract was documented with prominent root length inhibition and seed germination inhibition property i.e. 87.77±1.42, 66.93±0.90 and 49.17±0.75, 84.20± 0.70, 58.92±1.82 and 45.58±0.64 at concentration of 1000, 100, 10 µg/mL respectively among all other fractions. The Gt.Chf fraction was more phytotoxic as compared to other plant samples against radish seeds. Similarly, the root length inhibition by Gt.Eta was 86.33±1.53, 73.47±2.82 and 46.57±3.10 at concentration of 1000, 100 and 10 µg/mL andeffect of this fraction on the seed germination at concentration of 1000, 100 and 10 µg/mLwas 82.71±3.40, 65.52±0.93 and 39.49±1.12 shown in Table 1. Gt.Aqu has been shown with least effect on both parameters of the radish seeds. The inhibitory effect of the plantsamples is in order of Gt.Chf>Gt.Eta>Gt.Crd>Gt.Hex>Gt.Aqu.

Table 1: Phytotoxic effect of *G. tricorne*Stokes against radish seeds

S.No	Sample used	Concentration (µg/mL)	% root length inhibition (mean±SEM)	IC_{50} (µg/mL)	% germination inhibition (mean±SEM)	IC_{50} (µg/mL)
1	Gt.Crd	1000	81.78±2.52	31.21	77.25±3.63	72.62
		100	52.33±1.51		42.33±0.56	
		10	43.93±0.61		37.93±1.88	
2	Gt.Hex	1000	74.19±0.54	34.94	62.19±2.54	65.88
		100	60.46±1.78		50.31±1.78	
		10	38.99±0.84		42.73±0.84	
3	Gt.Eta	1000	86.33±1.53	12.20	82.71±3.40	25.54
		100	73.47±2.82		65.52±0.93	
		10	46.57±3.10		39.49±1.12	
4	Gt.Aqu	1000	47.67±0.58	186.00	41.77±0.26	47070

		100	33.70±3.51		27.72±1.65	
		10	25.90±1.03		19.28±0.39	
5	Gt.Chf	1000	87.77±1.42	12.65	84.20±0.70	21.36
		100	66.93±0.90		58.92±1.82	
		10	49.17±0.75		45.58±0.64	
6	PC	1000	91.23±1.54	0.023	89.46±2.55	2.05
		100	87.82±0.37		74.57±0.43	
		10	77.90±0.92		60.22±0.26	
7	NC	--	--	--	--	--

Key: Crd; Crude methanolic extract, Aqu; Aqueous fraction, Chf; Chloroform fraction, Hex; Hexane fraction,Gt;*Galium tricorne*,PC;+vecontrol (Paraquat), NC; -ve control, Data is represented as mean ± SEM.

Anthelmintic Assays

Round worms (*Ascaridia galli)*, adult earth worms (*Pheretima posthuma*) and tapeworms (*Raillietina spiralis*) were used to investigate anthelmintic potential of the Gt.Crdextract and solvent fractions of plant i.e, Gt.Chf, Gt.Hex, Gt.Eta, Gt.Bta and Gt.Aqu. Earthworms were used in this study because of their anatomical and physiological similarities with *Ascaris lumbricoides* of human intestinal round worms [32, 33]. The main indication for the collection of earth worm is the presence of small granules on the marshy soil. The earth worms were collected from the vicinity of COMSAT University and Abasyn University ChackShahzad, Islamabad Pakistan withaverage length of 8-9 cm. Similarly, round worms were obtained from the intestines of thefreshly slaughtered fowls with average length of 5-7 cm. Albendazole was used as a positive control. Paralysis and death time of the worms were noted and calculated.

Anthelmintic activity of the plant is given in the Figure1,2,3,4,5 & 6 showing that Gt.Crd and Gt.Chf fractions of *Galium tricorne* have better anti-helminthes activities.

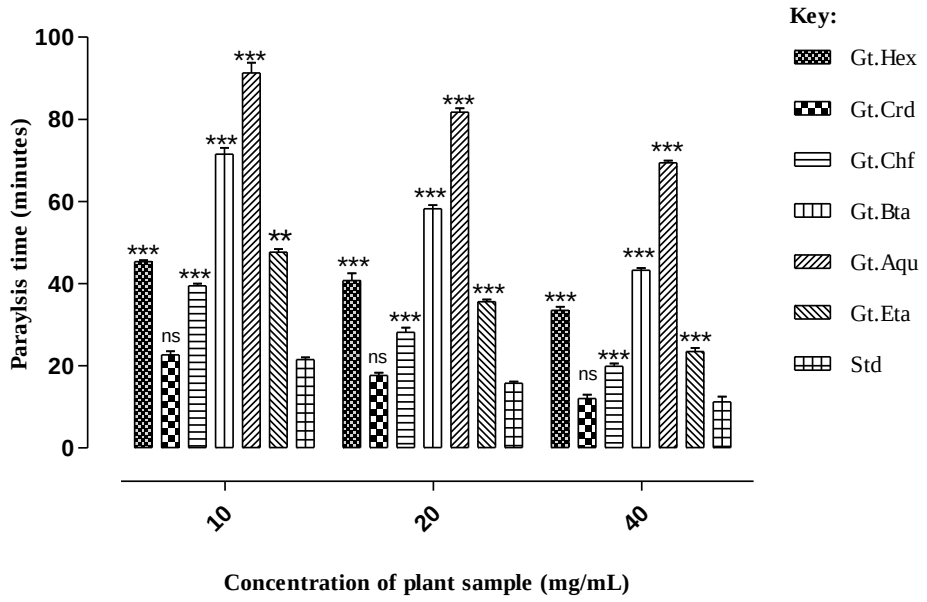

Figure 1: Paralysis time of *Pheretima posthuma* by *G. tricorne*

Keys: Aqu; Aqueous fraction: Crd; Crude methanolic extract: Bta; Butanolic fraction: Chf; Chloroform fraction: Eta; Ethyl acetate fraction: Hex; *n*-hexane fraction: Gt; *Galium tricorne*: Std: Albendazole; ns; non-significant: *; P<0.05: **; P<0.01: ***; P<0.001.

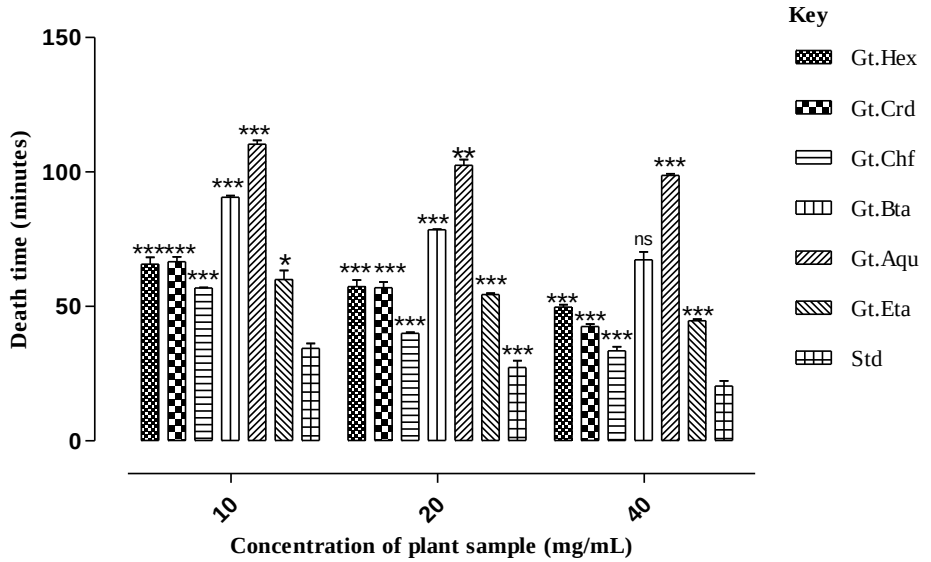

Figure 2: Death time of *Pheretima posthuma* by *G. tricorne*

Keys: Hex; *n*-hexane fraction: Chf; Chloroform fraction: Aqu; Aqueous fraction: Eta; Ethyl acetate fraction: Bta; Butanolic fraction: Crd; Crude methanolic extract: Gt; *Galium tricorne*: Std: Albendazole; ns; non-significant: *; P<0.05: **; P<0.01: ***; P<0.001.

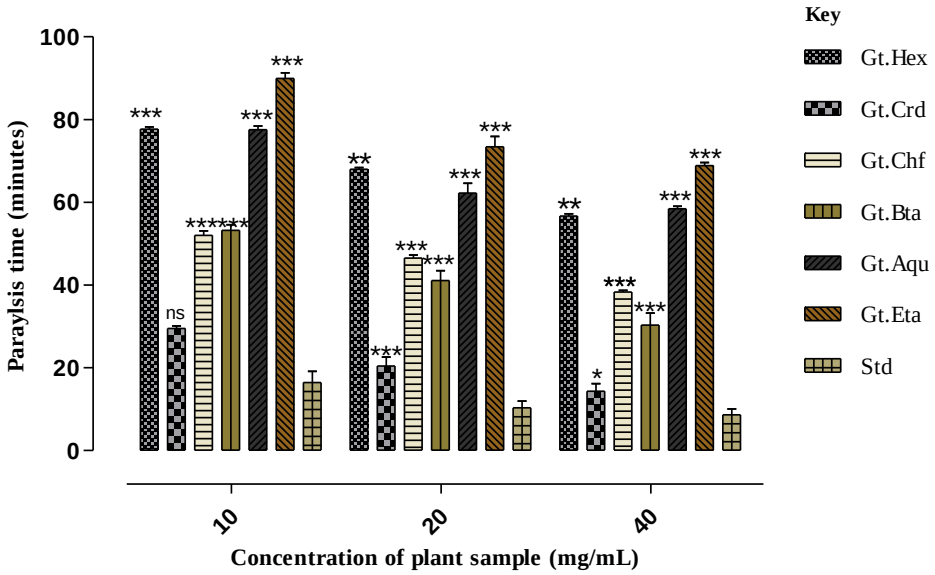

Figure 3: Paralysis of *Ascardiagalli* by *G. tricorne*

Key: Hex; *n*-hexane fraction: Chf; Chloroform fraction: Aqu; Aqueous fraction: Eta; Ethyl acetatefraction: Crd; Crude methanolic extract: Bta; Butanolic fraction: Gt; *Galium tricorne*: Std: Albendazole; ns; non-significant: *; P<0.05: **; P<0.01: ***; P<0.001.

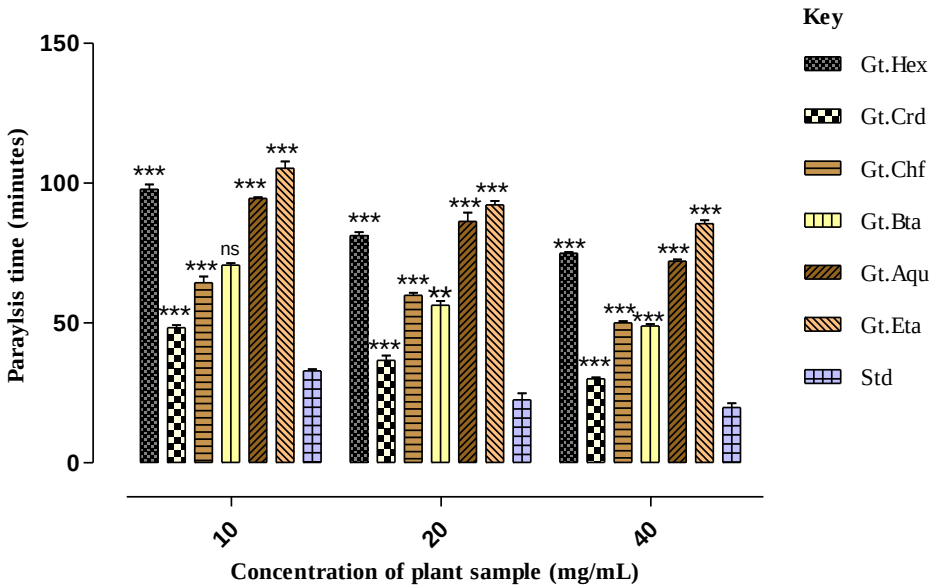

Figure 4: Death of *Ascardiagalli* by *G. tricorne*

Key: Hex; *n*-hexane fraction: Chf; Chloroform fraction: Aqu; Aqueous fraction: Eta; Ethyl acetatefraction: Crd; Crude methanolic extract: Bta; Butanolic fraction: Gt; *Galium tricorne*: Std: Albendazole; ns; non-significant: *; P<0.05: **; P<0.01: ***; P<0.001.

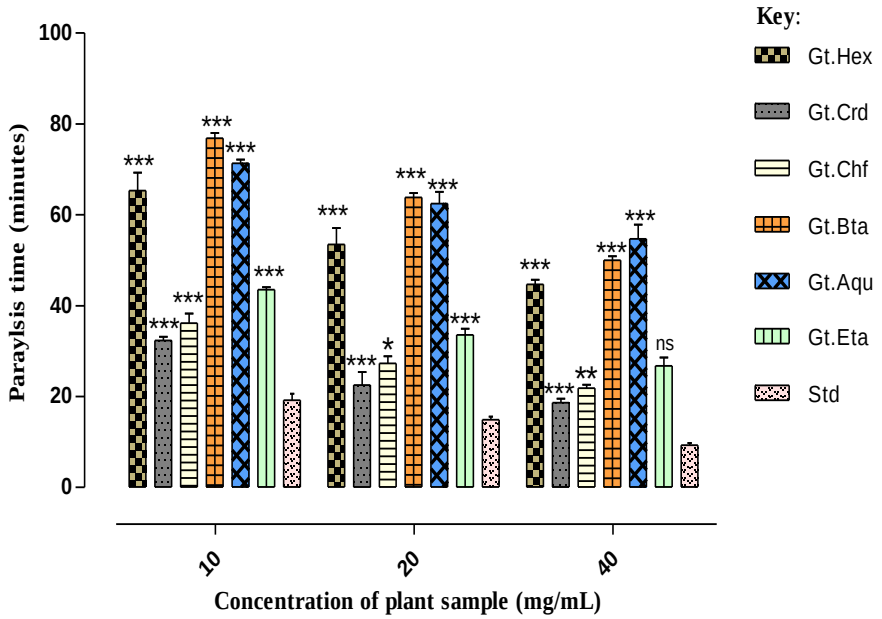

Figure 5: Paralysis time of *Raillientinaspiralis*by *G. tricorne*

Key: Hex; *n*-hexane fraction: Chf; Chloroform fraction: Aqu; Aqueous fraction: Eta; Ethyl acetatefraction: Crd; Crude methanolic extract: Bta; Butanolic fraction: Gt; *Galium tricorne*: Std: Albendazole; ns; non-significant: *; P<0.05: **; P<0.01: ***; P<0.001

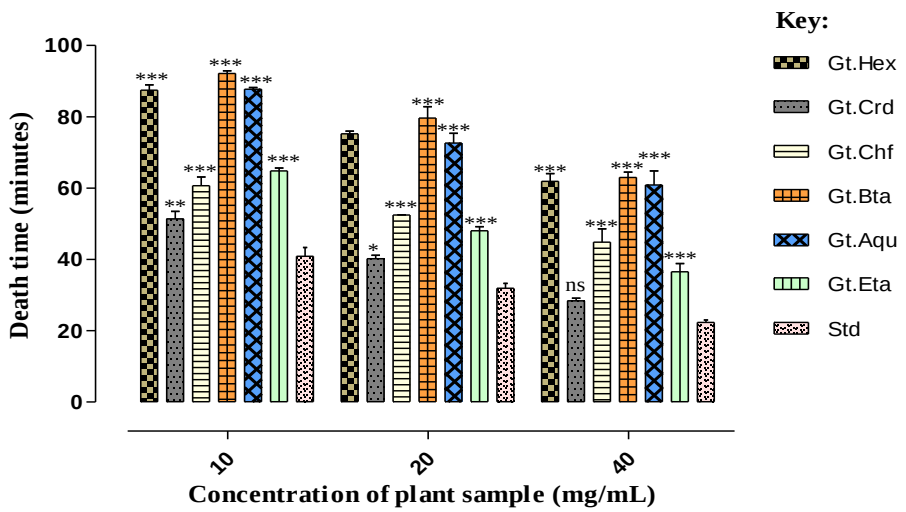

Figure 6: Death time of*Raillientinaspiralis*by *G. tricorne*

Key: Hex; *n*-hexane fraction: Chf; Chloroform fraction: Aqu; Aqueous fraction: Eta; Ethyl acetatefraction: Bta; Butanolic fraction: Gt; *Galium tricorne*: Crd; Crude methanolic extract: Std: Albendazole; ns; non-significant: *; P<0.05: **; P<0.01: ***; P<0.001.

GC-MS Spectrum of Ethyl Acetate Fraction

Twenty two (22) different compounds were identified through GC-MS technique in ethyl acetate fraction of *Galium tricorne* shown in Figure 7 and Table 2. Bioactive compounds sorted out on the basis of literature are shown in Figure 8.

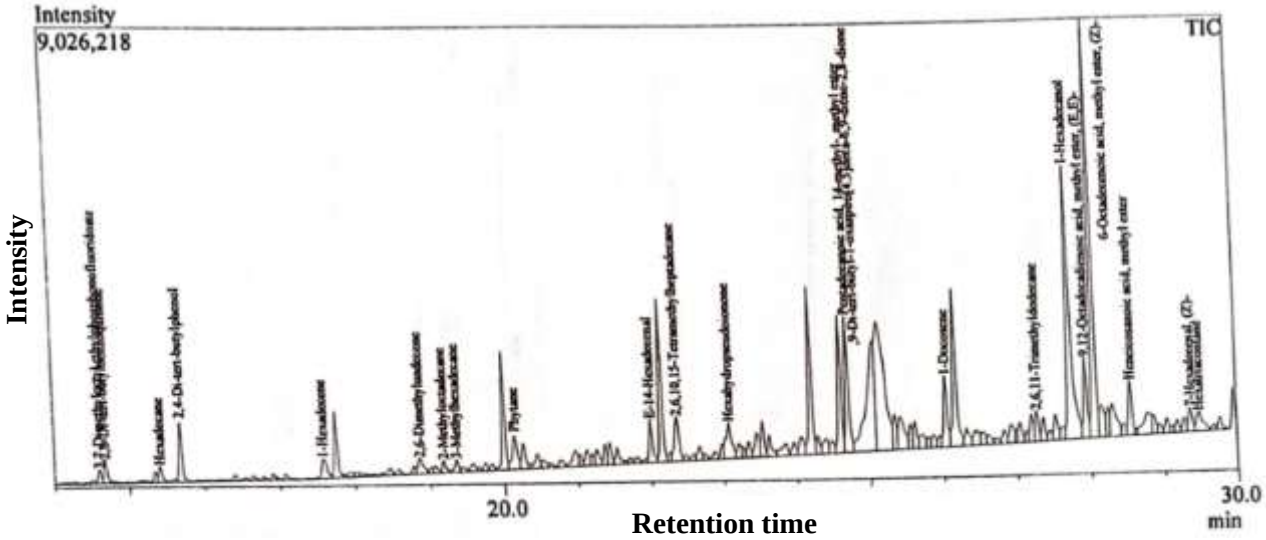

Figure 7:GC-MS Spectrum of ethyl acetate fraction of *Galium tricorne* Stokes

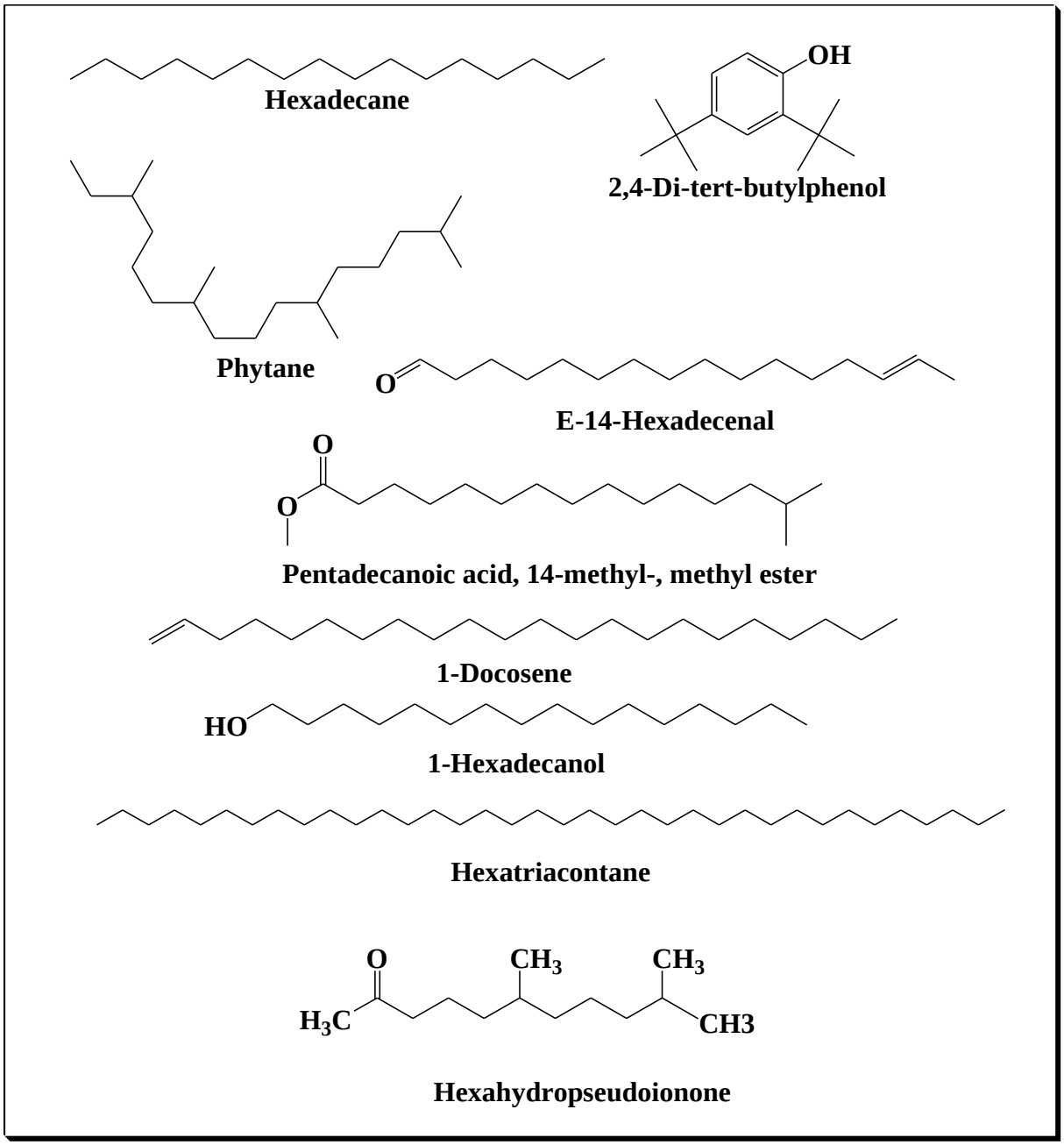

Figure 8: Structural formula of bioactive compounds identified in ethyl acetate fraction of *Galium tricorne* Stokes.

Table 2: Compounds identified in ethyl acetate fraction of *Galium tricorne*

ID#	Name of Identified Compounds	Retension Time	Area	Concentration (%)
1	3,7-Dimethyloctyl ethylphosonofluoridoate	14.598	153239	1.36
2	2,6-Di-tert-butylbenzoquinone	14.675	110328	0.98
3	Hexadecane	15.405	182749	1.62
4	2,4-Di-tert-butylphenol	15.679	881883	7.83
5	1-Hexadecene	17.572	106416	0.94
6	2,6-Dimethylundecene	18.861	342571	3.04
7	2-Methyloctadecane	19.182	117147	1.04
8	3-Methylhexadecane	19.353	118174	1.05
9	Phytane	20.139	438874	3.90
10	E-14-Hexadecenal	21.995	236644	2.10

11	2,6,10,15-Tetramethyl heptadecane	22.356	685968	6.09
12	Hexahydropseudoionone	23.073	564544	5.01
13	9-Di-tert-butyl-1-oxaspiro[4.5] deca-6,9-diene-2,8-dione	24.607	665139	5.91
14	Pentadecanoic acid, 14-methyl-, methyl ester	24.701	1310534	11.64
15	1-Docosene	26.036	354610	3.15
16	2,6,11-Trimethyldodecane	27.264	263759	2.34
17	1-Hexadecanol	27.724	1769815	15.72
18	9,12-Octadecadienoic acid, methyl ester, (E,E)-	27.929	377302	3.35
19	6-Octadecenoic acid, methyl ester, (Z)-	28.048	1753516	15.57
20	Heneicosanoic acid, methyl ester	28.538	513682	4.56
21	7-Hexadecenal, (Z)-	29.365	92957	0.83
22	Hexatriacontane	29.470	221644	1.97

DISCUSSION

Natural products have an important role in treating different types of human illnesses. The main constituents of herbal remedies are natural compounds. World Health Organization (WHO) estimated that 70% of world population depends on phyto-medicines for primary health care needs. These natural products give rise to lead compounds having definite efficacy, quality and safety profile. They are mostly established in developing countries, for the isolation of drugs in recent years [39].

It has been shown that aqueous extract of species of *Rubiaceae* family have phytotoxic potential on the growth and germination of other species of plants [40, 41]. Most of the phenolic compounds are responsible for the phytotoxicity nature of the plant [31]. These phytochemical or allelochemicals are found in different parts of the plants including bark, roots, seeds, leaves and rhizomes, but in all parts of the single plant species it may varies [42]. By exposing the seeds of vulnerable plant species to allelochemicals germination inhibition, seedlings inhibition, reduction in growth and occurrence of abnormal metabolism [42, 43, 44, 45]. *Galium tricorne* Stokes has been documented with cytotoxicity, antibacterial and anticancer (MTT assay) potential [8].

*Galium tricorne*has been evaluated for the anthelmintic potential. Other membersof the *Rubiaceae* family namely *Neolamarckiacadamba* also have prominent antifungalactivity [34]. *Wendlandia thyrsoidea* (*Rubiaceae*) leaves extract was documented with anthelmintic activity against adult earthworm's *Pheretima posthuma* [35]. Similarly,*Ixora coccinea* chloroform extract having good anti-helmintic activity [36]. Anti-helmintic activity was also observed in leaves extract of *Ixora cibdela* Craib (*Rubiaceae*) [37]. The plant *Gardenia gummifera* L. F. (*Rubiaceae*) was documented with anthelmintic property [38]. Different compounds have been identified in the seeds, crude methanolic extract and chloroform fraction through GC-MS technique of *Galium tricorne* Stokes [8].

In the current study it has been documented that *Galium tricorne* Stokes have prominent anthelmintic effect against worms and phytotoxic potential against radish seed germination and root length inhibition. Besides these 22 different compounds were identified in ethyl acetate fraction of the plant and some of the compound were sorted out on the basis of bioactivity.

2,4-Di-tert-butylphenol or 2,4-bis(1,1-dimethylethyl)-phenol (2,4-DTBP) is a common natural lipophilic phenolic compound exhibits potent toxicity potential almost all the testing organisms having prominent antioxidant, anti-inflammatory, cytotoxicity against HeLa cells, Insecticidal, nematicidal, antibacterial, antiviral, antifungal andphytotoxicity activities [46]. The compound hexahydropseudoionone has been used in cosmetic like, perfumes[47]. Likewise, Hexatriacontane having free radical scavenging potential [48, 49].1-Docosene is also commonly used in producing grease, lubricants, fuels, lubricant additivesand fuel additives[50]. The red seaweed *Corallina officinalis* dichloromethane extract also contains 1-docosene having antibacterial effect [51]. The compounds E-15-heptadecenal and E-14-hexadecenal were obtained from the fungus *Chaetomium globosum* which displayed antiproliferative effect against cancer cell lines [52]. The fatty alcohol 1-

Hexadecanol possessemulsifier, antioxidant, thickening agent, opacifier and emollient properties [53]. The phyto-compound Pentadecanoic acid, 14-methyl-, methyl ester having analgesic and antitumor potential [54]. Similarly the compound Hexadecane exhibited antibacterial, free radical scavenging and antifungal potentials [55, 56].
The most commonly isoprenoid hydrocarbons biomarkers found in the geosphere are phytane (2,6,10,14-tetramethylhex-adecane) and pristane (2,6,10,14-tetrame-thylpentadecane) [57].

CONCLUSION

It has been concluded that for the first time phytotoxicity assay, anthelmintic assay and identification of compounds in ethyl acetate fraction of *Galium tricorne* has been documented. From the biological assays it may also be inferred that the plant contains different allelochemical and anthelmintic secondary metabolites. The current findings of the research also substantiate the scientific background for the ethnomedicinal uses of *Galium triocrne*. These findings emphasize that the cytotoxic and anthelmintic potential of the plant might be due to the presence of bioactive compounds.

Conflict of interest

No conflicts of interest are concealed by the authors.

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References

1. Khan S, Gul M: In vitro antifungal activity of *Rhazyastricta*. Pak J Pharm Sci 2007, 20(4):274–279.
2. Ahmad M, Sultana S, Hadi S, Hadda T, Rashid Zafar M, Khan M, Khan M, Yaseen G: An ethnobotanical study of medicinal plants in high mountainous region of Chail valley (District Swat- Pakistan). J EthnobiolEthnomed 2014, 10:36.
3. Abbasi A, Khana MA, Mushtaq A, Muhammad Z, Sarwat J, Shahzia S: Ethno-pharmacological application of medicinal plants to cure skin diseases and in folk cosmetics among the tribal communities of North-West Frontier Province. Ethnopharmacol 2010, 128:322–335.
4. Soza, V. L., & Olmstead, R. G. (2010). Molecular systematics of tribe *Rubieae* (*Rubiaceae*): Evolution of major clades, development of leaf–like whorls, and biogeography. *Taxon, 59*(3), 755-771.
5. Ergun, F., Deliorman, D., Velioglu, A., Sener, B., & Bozok Johansson, C. (1999). Anti-microbial activities of *Galium* species. *Eczacilik Fakultesi Dergisi-Gazi Universitesi, 16*(7-12).
6. Temizer, H., & Şene, B. (1996). Determination of total plavonoid in varioljs Galium species by differential pulse polarography. *J. Fac. Pharm. Gazi*, *13*(1), 97-103.
7. Mohsin, R., & Choudhary, M. I. (2000). Medicinal plants with anticonvulsant activities. *Studies in Natural Products Chemistry, Elsevier,22*, 507-553.
8. Khan, S. A., Khan, H., Ahmad, S., Rehman, F. U., Khan, A. A., & Khan, M. A. (2022). GCMS characterization and biological potential of the seeds and aerial part of *Galium tricorne* Stokes. *Brazilian Journal of Biology*, *84*, e256920.
9. Geary TG, Woo K, McCarthy JS, Mackenzie CD, Horton J, Prichard RK, de Silva NR, Olliaro PL, Lazdins-Helds JK, Engels DA: Unresolved issues in anthelmintic pharmacology for helminthiases of humans. Int J Parasitol2010, 40(1):1–13.
10. Blumenthal DS, Schultz MG: Incidence of intestinal obstruction in children infected with Ascarislumbricoides. Am J Trop Med Hyg 1975, 24(5):801.

11. Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A, Diemert D, HotezPJ: Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet 2006, 367(9521):1521–1532.
12. Ivory C, Chadee K: DNA vaccines: designing strategies against parasitic infections. Genet Vaccine Ther 2004, 2(1):17.
13. Dhar D, Sharma R, Bansal G: Gastro-intestinal nematodes in sheep in Kashmir. Vet Parasitol 1982, 11(2):271–277.
14. Coles GC: Nematode control practices and anthelmintic resistance on British sheep farms. Vet Rec 1997, 141(4):91–93.
15. Liu LX, Weller P: Antiparasitic drugs. N Engl J Med 1996, 334(18):1178.
16. Ali N, Shah S, Shah I, Ahmed G, Ghias M, Imran K: Cytotoxic and anthelmintic potential of crude saponins isolated from *AchilleaWilhelmsii* C. Koch and *TeucriumStocksianum*boiss. BMC Complement Altern Med 2001, 11(1):106.
17. Akhtar M, Iqbal Z, Khan M, Lateef M, Bachman D: Anthelmintic activity of medicinal plants with particular reference to their use in animals in the Indo-Pakistan subcontinent. Small Ruminant Res 2000, 38(2):99–107.
18. Khuda F, Iqbal Z, Zakiullah, Khan A, Nasir F, N M: Metal analysis, phytotoxic, insecticidal and cytotoxic activities of selected medicinal plants of Khyber Pakhtunkhwa. Pak J Pharm Sci 2012, 25(1):51–58.
19. WHO: Evaluation of Certain Food Additives and Contaminants, WHO Technical Report Series 776. Geneva: World Health Organization; 1989.
20. Zeb A, Sadiq A, Ullah F, Ahmad S, Ayaz M: Phytochemical and toxicological investigations of crude methanolic extracts, subsequent fractions and crude saponins of *Isodonrugosus*. Biol Res 2014, 47:57.
21. Osadebe, P., & Okoye, F. (2003). Anti-inflammatory effects of crude methanolic extract and fractions of *Alchornea cordifolia* leaves. *Journal of Ethnopharmacology*, *89*(1), 19-24.
22. Turker, A. U., & Camper, N. (2002). Biological activity of common mullein, a medicinal plant. *Journal of Ethnopharmacology*, *82*(2-3), 117-125.
23. Parida, S., Patro, V. J., Mishra, U. S., Mohapatra, L., & Sannigrahi, S. (2010). Anthelmintic potential of crude extracts and its various fractions of different parts of *Pterospermum acerifolium* Linn. *Inter J Pharma Sci Rev Res*, *1*(Suppl 2), 107-111.
24. Dash, G. K., Suresh, P., Sahu, S., Kar, D., Ganapaty, S., & Panda, S. (2002). Evaluation of *Evolvulus alsinoides* Linn. for anthelmintic and antimicrobial activities. *Journal of Natural Remedies*, *2*(2), 182-185.
25. Kaushik, R., Katiyar, J., & Sen, A. (1974). Studies on the mode of action of anthelminticswith *Ascaridia galli* as the test parasite. *The Indian Journal of Medical Research*, *62*(9), 1367-1375.
26. Hassouni, T., & Belghyti, D. (2006). Distribution of gastrointestinal helminths in chicken farms in the Gharb region—Morocco. *Parasitology Research*, *99*(2), 181-183.
27. Lal, J., Chandra, S., Raviprakash, V., & Sabir, M. (1976). *In vitro* anthelmintic action of some indigenous medicinal plants on *Ascardia galli* worms. *Indian journal of Physiology and Pharmacology*, *20*(2), 64-68.
28.Gbolade, A., & Adeyemi, A. (2008). Investigation of *in vitro* anthelmintic activities of *Pycnanthus angolensis* and *Sphenocentrum jollyanum*. *Fitoterapia*, *79*(3), 220-222.
29. Stein, S., Mirokhin, D., & Tchekhovskoi, D. (2002). The NIST Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectra Library; Standard Reference Data Program of the National Institute of Standards and Technology. *Gaithersburg, MD, USA*.
30. Adams, R. (2007). *Identification of essential oil components by gas chromatography/ mass spectrometry*. Allured Publishing: Carol Stream, IL, USA, pp. 804.
31. Inderjit. (1996). Plant phenolics in allelopathy. *The Botanical Review*, 186-202.

32. Akhtar, M. S., Iqbal, Z., Khan, M., & Lateef, M. (2000). Anthelmintic activity of medicinal plants with particular reference to their use in animals in the Indo–Pakistan subcontinent. *Small Ruminant Research, 38*(2), 99-107.
33 Ali, N., Shah, S. W. A., Shah, I., Ahmed, G., Ghias, M., & Khan, I. (2011). Cytotoxic and anthelmintic potential of crude saponins isolated from *Achillea Wilhelmsii* C. Koch and *Teucrium Stocksianum* boiss. *BMC Complementary and Alternative Medicine, 11*(1), 1-7.
34. Islam, T., Das, A., Shill, K. B., Karmakar, P., Islam, S., & Sattar, M. M. (2015). Evaluation of membrane stabilizing, anthelmintic, antioxidant activity with phytochemical screening of methanolic extract of *Neolamarckia cadamba* fruits. *Journal of Medicinal Plants Research, 9*(5), 151-158.
35. Sindhe, M. A., Bodke, Y. D., &Kenchappa, R. (2015). *In vitro* Anthelmintic activity of *Wendlandiathyrsoidea* leaves extracts against *Pheretima posthuma*. *International Journal of Pharmacology, 2*(6), 1-4.
36. Surana, A., Aher, A., Pal, S., & Deore, U. (2011). Evaluation of anthelmintic activity of *Ixora coccinea*. *Int. J. Pharm. Lif. Sci, 6*(2), 813-814.
37. Buathong, R., Schindler, F., Schinnerl, J., Valant-Vetschera, K., Bacher, M., Potthast, A., Rosenau, T., & Vajrodaya, S. (2019). Uncommon fatty acids, Iridoids and other secondary metabolites from the medicinal plant species *Ixora cibdela* Craib (*Rubiaceae*). *Phytochemistry Letters, 33*(77-80.
38. Sen, S., Chakraborty, R., Sridhar, C., Reddy, Y., & De, B. (2010). Free radicals, anti-oxidants, diseases and phytomedicines: current status and future prospect. *International Journal of Pharmaceutical Sciences Review and Research, 3*(1), 91-100.
39. Tangkiatkumjai M, Boardman H, Walker D-MJBCM, Therapies. Potential factors that influence usage of complementary and alternative medicine worldwide: a systematic review. 2020;20(1):115.)
40. Frescura VDS, Kuhn AW, Laughinghouse IV HD, Nicoloso FT, Lopes SJ & Tedesco SB (2013) Evaluation of the allelopathic, genotoxic, and anti-proliferative effect of the medicinal species *Psychotriabrachypoda* and *Psychotriabirotula* (*Rubiaceae*) on the germination and cell division of *Eruca sativa* (*Brassicaceae*).*Caryologia*, 66:138-144.
41. Oliveira AKMD, Matias R, Lopes SS &Fontoura FM (2014b) Allelopathy and influence of leaves of *Palicourearigida* (*Rubiaceae*) on seed germination and seedling formation in lettuce. Bioscience Journal, 30:938-947.
42. GATTI, A. B.; FERREIRA, A. G.; ARDUIN, M.; PEREZ, S. C. J. G. A. Allelopathic effects of aqueousextracts of *Aristolochiaesperanzae* O. Kuntze on development of *Sesamumindicum*L. seedlings. ActaBotânicaBrasílica, São Paulo, v. 24, n. 2, p. 454-461, 2010.
43. GUSMAN, G. S.; YAMAGUSHI, M. Q.; VESTENA, S. Potencialalelopático de extratosaquosos de Bidenspilosa L., *Cyperusrotundus* L. e *Euphorbia heterophylla* L. Iheringia, SérieBotânica, Porto Alegre, v. 66, n.1, p. 87 - 98, 2011.
44. GRISI, P. U.; GUALTIERI, S. C. J.; RANAL, M. A.; SANTANA, D. G. Influênciaalelopática do extratoaquoso de raiz de *Sapindussaponária* L. sobrecapim-arroz e corda-de-viola. Bioscience Journal, Uberlândia, v. 29, n. 3, p. 760-766, 2013.
45. SILVESTRE, D.M.; KOLB, R.M.; FREI, F.; SANTOS, C. Phytotoxicity of organic extracts of *Turneraulmifolia* L.and *Turnera diffusa*Willd.ex Schult. in cucumber seeds. Acta Botanica Brasilica, Feira de Santana, v. 27, n. 3, p. 476-482, 2013.
46. Zhao, F., Wang, P., Lucardi, R. D., Su, Z., & Li, S. (2020). Natural sources and bioactivities of 2, 4-di-tert-butylphenol and its analogs.*Toxins, 12*(1), 35**).**
47. Shah, Z., Badshah, S. L., Iqbal, A., Shah, Z., Emwas, A. H., &Jaremko, M. (2022). Investigation of important biochemical compounds from selected freshwater macroalgae and their role in agriculture. *Chemical and Biological Technologies in Agriculture*, *9*(1), 9.
48. Arora, S., Kumar, G., &Meena, S. (2017). GC-MS analysis of bioactive compounds from the whole plant hexane extract of *Cenchrussetigerus*Vahl. *Pharma Science Monitor, 8*(4), 137-46.

49. Igwe, O. U., &Offiong, S. P. (2015). Chemistry of semiochemicals used as trail pheromones in tropical fire ant (*Solenopsisgeminata*). *International Journal of Chemical and Biochemical Sciences*, *7*, 35-40.
50. Adeyinka, O. A., Bankole, I. A. S., & John, R. (2022). Gc-Ms Bioprospecting Of Phytochemicals In The Ethanolic Extract Of Fresh *Mangiferaindica* LEAVES. *Journal of Chemical Society of Nigeria*, *47*(4).
51. SAHAR, W.M.H. and AIDA, H.S., 2018. GC/MS identification and applications of bioactive seaweed extracts from Mediterranean coast of Egypt. The Egypt Journal of Aquatic Research, vol. 22, no. 5, pp. 1-21.
52. Kaur, N.; Arora, D.S.; Kalia, N.; Kaur, M. Bioactive potential of endophytic fungus *Chaetomium globosum* and GC-MS analysis of its responsible components. Sci. Rep. 2020, 10, 18792.
53. Amudha, P., Jayalakshmi, M., Pushpabharathi, N., &Vanitha, V. (2018). Identification of bioactive components in Enhalusacoroides seagrass extract by gas chromatography-mass spectrometry. *Asian Journal of Pharmaceutical and Clinical Research*, *11*(10), 313-315.
54. Abirami, P., Gomathinayagam, M., &Panneerselvam, R. (2012). Preliminary study on the antimicrobial activity of *Enicostemmalittorale* using different solvents.*Asian Pacific journal of tropical medicine*, *5*(7), 552-555.
55. Yogeswari, S., Ramalakshmi, S., Neelavathy, R., &Muthumary, J. Y. (2012). Identification and comparative studies of different volatile fractions from *Monochaetiakansensis* by GCMS.*Global Journal of Pharmacology*, *6*(2), 65-71.
56. Hsouna, A. B., Trigui, M., Mansour, R. B., Jarraya, R. M., Damak, M., &Jaoua, S. (2011). Chemical composition, cytotoxicity effect and antimicrobial activity of *Ceratoniasiliqua* essential oil with preservative effects against Listeria inoculated in minced beef meat. *International journal of food microbiology*, *148*(1), 66-72.
57. Volkman, J. K. (1986). Acyclic isoprenoids as biological markers.*Biological markers in the sedimentary record*, 1-42.