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Pharmacognostic and pharmacological studies of leaf, stem and fruit of emex spinosa (L.) Campd

Samin Jan[1], Sher Wali[2], Nadeem Ahmad[3], Izhar Ahamd[1], Muhammad Hamayun[4], Naila Shah[1]

[1]*Department of Botany, Islamia College Peshawar, Pakistan*

[2]*Department of Botany, Shaheed BB University Sheringal, Dir (U), Khyber Pakhtunkhwa, Pakistan*

[3]*Department of Botany, University of Peshawar, Pakistan*

[4]*Department of Botany, Abdul Wali Khan University, Mardan, Pakistan*

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Abstract

The current study deals with the pharmacognostic and pharmacological assessment of *Emex spinosa* (L.) Campd. The macro studies of leaf showed reticulate venation, anatomically the surface showed stomata with paracytic pattern, polygonal epidermal cells on adaxial surface, single layered palisade cells and vascular bundles. Multicellular trichomes and perismatic crystals were recorded in the powder drug. The transverse section of the stem showed epidermis, cortex, cork, metaxylem, protoxylem, medulla and phloem. Phytochemical investigation revealed the presence of carbohydrates, proteins, phenols, alkaloids, triterpenoids and flavonoids. The total flavonoids content was 15.08 mg/g in stem, 58.66 mg/g in leaf and 37.39 mg/g in fruit. The total phenolic content varied was 77.84 mg/g in stem, 97.09 mg/g in leaf and 97.72 mg/g in fruit. The highest percent value for free radical scavenging activity against 2,2-diphenyl-2-picrylhydrazyl (DPPH), was shown by fruit extract 90.45%, followed by leaf 79.32% and lowest by stem 48.33%. The acute toxicity test of the crude extract was safe up to the dose of 50 mg/kg. The leaf extract showed significant cytotoxic activity at concentration of 500 mg/ml against brine shrimps (80%), stem extract showed low activity. Fruit Atropine sulphate extracts produced highest, 55.94%, inhibition at a dose of 2 mg/kg whereas the fruit methanolic extract showed the highest percent inhibition (24.4%) at a dose of 10 mg/kg. Phytotoxic activity in all the plant extracts was moderate at and low at concentrations of 1000µg/ml and 10 µg/ml respectively.

* **Corresponding Author:** Sher Wali, Email ✉ sherwali_icup@yahoo.com

Introduction

Polygonacae is a group of morphologically different herbs, shrubs, small trees or climbers characterized by simple leaves with covering ochrear stipules, unilocular ovary and endospermic seeds (Hutchinson & Dalziel, 1954; Brummitt, 1992). Fruits in family Polygonacae are achenes, excluding its native habitat and Australia, E. *spinosa* occupies highly upset areas, occurring along railway tracks, roads and areas of cereal cultivation in upland Kenya, pyramids of Giza and in waste sandy places (Graham, 1958; Siddiqi, 1973). The male flowers, together with the occasional perfect flower, form in short axillary racemes, often emerging between the female achenes (Zohary, 1966). Leaves are cauline, brown membranous ochreae are present at the base of the petioles. The spiny female flowers are sessile and formed in clusters in the axils of the leaves forming first on the crown about a month after the seed has germinated and while the plant is still a new rosette (Evenari *et al*., 1977). Some species belonging to the family polygonaceae grow beside the edge of drains in India (Varma *et al*., 1984). Polygonaceae is a diverse family containing about 1,200 species from 48 genera, represented by 19 genera and 103 species (Qaisar, 2001; Sanchez & Kron, 2008 and Freeman & Reveal 2005). *Emex spinosa* is regarded as a minor weed and plays an important role in providing fuel for local inhabitants, forage source for animals, and have potential medicinal values (Holm *et al*., 1979; Thalen, 1979). Its phytochemical screening revealed the presence of anthraquinones, alkaloids, coumarins and flavonoides (Rizk, 1986). *Emex spinosa* is edible by the local inhabitants who plucked it and eat its petiole and carrot-like tap root (Mandeville and Mullens, 1990). *Emex spinosa* (L.) Campd. an annual member of family Polygonaceae, is a Mediterranean weed (Boulos & El- Hadidi 1994). *Emex spinosa* is one of the important medicinal plants used to stimulate appetite, relief dyspepsia, colic and a remedy for stomach disorders. It is believed to be diuretic and purgative, the young leaf has been used as spinach (Watt & Breyer-Brandwjik 1996). Thirteen compounds were isolated from the aerial parts of *Emex spinosa* growing in Egypt (Kader *et al.,* 2006). A correlation between radical scavenging activities of extracts with total phenolic content was showed that large amounts of phenolic compounds may contribute towards the anti-inflammatory and antioxidant properties (Shankar *et al*., 2008). The comparative study between the nutritive values of *Emex spinosa* described it to be a capable fodder plant (Shaltout *et al*., 2009). Animals treated with ethanolic extracts of *E. spinosa*, *H. salicornicum*, *L. pyrotechnica*, and *O. baccatus* showed major improvement of the relative weight of reproductive organs, sperm motility, sperm count and total abnormality of sperm (Gamal *et al*., 2012). The complete plant of *Viola betonicifolia* led to the segregation of 4-hydroxyl cumarin (4HC) which exhibited considerable safety profile in acute toxicity test (Naveed *et al*., 2012). Under different conditions *E. spinosa* have the ability to germinate but percentage germination that will different under different ecological conditions (Shoab *et al*., 2012). Aloe-emodin glucoside and four fractions from *Emex spinosa* were evaluated for their cytotoxic and antimicrobial activities, different fraction showed significant effect (Raheim *et al*., 2014).

The current research work was carried to evaluate the Pharmacognostic and Pharmacological values of Leaf, Stem and Fruit of *Emex spinosa* (L.) Campd. Literature revealed that *Emex spinosa* is an important medicinal plant so the current work was designed to know the plant details regarding the morphological features, organoleptic and phytochemical analysis and pharmacognostic and pharmacological properties.

Materials and methods

Pharmacognostic study

Mature plants of *E. spinosa* were collected in the flowering stage in the month February, 2013. Few fresh plants were used for morpho-anatomical studies. The plants were shade dried at room temperature. A specimen of the plant was properly pressed and mounted on herbarium sheet and preserved in herbarium as a voucher specimen. The dried leaves, stems and fruits of the plant were powdered separately in an electric grinder and were

placed in air tight jars for powder drug studies and methanolic extraction for various bioassays. Macroscopic studies including morphological features and organoleptic evaluation of the drug of leaves (Shape, Size, Odor, Taste, Venation, Apex, Type, Leaf margin, Color, Surface, and Phyllotaxis, Texture) and stem (Length, Odor, Taste, Type, Color , Surface and Internode length) were carried out following the method of Trease and Evans (2002). Stomata type (upper and lower surface), Stomata index (upper and lower surface) and Stomata number (upper and lower surface) were studied according to the method prescribed by Trease and Evans (2002). Powdered drug study was carried following Wallis (2005).

Preliminary phytochemical qualitative tests

For the detection of phytocontituents different qualitative tests of methanolic extract of *E. spinosa* were carried out. For carbohydrates detection, Felling's test and Benedicts test (Evans, 2002) were carried out. For alkaloids detection Wanger's test was performed (Khandelwal, 2004). Proteins detection was done with Biuret test (Gahan, 1984). While Phytosterols detection was done through Salkowski's test (Harborne, 1998). For Flavonoids detection, Alkaloid reagent test was perforemed. Glycosides detection test was performed by by treating 500mg of extract was with 2ml acetic acid and then 1 drop of $FeCl_3$ and last with H_2SO_4. The appearance of brown ring color at interphase indicated the presence of glycosides.

Chemical evaluation

Aluminum chloride colorimetric method was used for determination of total flavonoids (Chang *et al*., 2002). Each plant extracts (0.5 ml of 1:10 g ml-1) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. The total phenolic content was determined according to Velioglu *et al.* (1998) using the Folin-Ciocalteau reagent. 1.5ml of this reagent (10%) was mixed with each extract. After 3min, 1.5ml of sodium carbonate (6%) were added. The absorbance was measured after 1H of incubation at 760nm against a blank.

Dpph free radical scavenging assay

To evaluate the capability of prepared extracts to scavenge free radical DPPH° (α, α-diphenyl-β-picrylhydrazyl) using the method of (Amarowicz *et al.* 2004). The antiradical effect of the extracts on DPPH was determined according to Brand-Williams *et al.* (1995) method. Different concentrations of the extracts have been studied: pure, 1/2, 1/3 and 1/4. The concentration efficiency EC50 and EA (1/Ec50) values have been determined for each extract. Results are expressed as percentage mg /ml.

Total antioxidant capacity
(Phosphomolybdenum method)

Total antioxidant capacity was determined by phosphomolybdenum method (Prieto *et al*., 1999). The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid ρH. A 0.3 ml extract was combined with 3 ml of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer (Jenway 6025) against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract was used as the blank. The antioxidant activity is expressed as the number of gram equivalent of ascorbic acid.

Pharmacological evaluation

Bioassays included the following aspects:

Phytotoxic bioassay

Lemna minor phytotoxicity test was carried out in order to know phytotoxic potential of *E. spinosa* following the method of Atta-ur-Rehman (2001). Percentage inhibition was calculated by formula, Percentage inhibition = 100 – (no. of fronds in test sample/ no. of fronds in negative control) × 100.

Cytotoxic bioassay

In order to determined cytotoxic potential of *E. spinosa*, the extract of stem, leaf and fruit was used against brine shrimps following the method of Atta-ur-Rehman *et al*., (2001). Acute toxicity test of *E.*

spinosa was carried out against mice following Naveed *et al.,* (2013).

Antispasmodic bioassay

The antispasmodic activity of *E.spinosa* was carried out against mice following Shamkuwar *et al.,* (2012). Peristaltic index was calculated using formula, Peristaltic index = distance travelled by charcoal meal / Total length of small intestine × 100.

Results

Morphological and anatomical characteristics.

The morphologic characters of *Emex spinosa* showed that *Emex spinosa* is an annual herb about 32cm tall, flowering season of the plant starts from the mid of February to April. Leaves were lanceolate with acute apex having deep incisions, venation reticulate, upper surface was dark green and lower surface was light green in color. Leaf length 13-17cm, width 1.5-2.5cm, has bitter taste and indistinct odor. Stem color light green, hairy upright cylindrical. Root small taproot, brown in color, with secondary rootlets directed vertically downwards, bitter in taste and indistinct in odor. Flowers yellow in color, obovate, calyx green, corolla yellow and androecia numerous. Fruit achene, obovoid in shape indistinct odor and bitter in taste (Table No. 1). For the correct identification of various features of plant like the internal structure (microscopy) plays important role (Nancy and Dengler, 2002). Powder drug study of *Emex spinosa* leaf showed star shaped trichome, non-glandular multicellular 6-7 celled trichome, stomata with epidermal cells, starch grains, stomata anisocytic, epidermal cells with palisade cells and multicellular trichomes, fragments of epidermal cells, vessels attached with paranchymatous cells, fibers from vein with paranchymatous cells, spongy mesophyll cells (Figure No. 1). Organoleptic study showed that leaf powder drug has dark green color, slightly pungent and bitter in taste. Stomatal study revealed Hemiparacytic stomata, characterized by single parallel cells (Carpenter, 2005); Anomocytic characterized by four or more undifferentiated cells (Metcalfe & Chalk, 1957); and Anisocytic, stomata surrounded by three unequal sized subsidiary cells, to the longitudinal axis the wall is at right angle to the stomata (Figure No.1). Stomatal index of upper epidermis was 22.24 – 26.45 (24.34±0.605), and stomatal index of lower epidermis was 32.58 – 37.51 (35.04±0.590) (Table No. 2). Leaf transverse section of *Emex spinosa* showed that width of upper epidermis ranges from 25µm to 32µm while diameter of lower epidermis ranges from 27-30µm in. The upper epidermis has multicellular non-glandular trichome 8-12µm in thickness. Palisade parenchyma cylindrical in shape was single layered; cells were 12.5-17.5µm in length and 1.5-2.00µm in width. Spongy mesophyll cells rounded to oval or elongated in shape with 2-4µm in length and 1.5-2.5µm in width. Upper epidermis has less number of stomata (24.34±1.22) as compare to lower epidermis (35.04±0.96). Lower epidermal cells were 3.5-5.6µm in length and 1.5-2.5µm in width, elongated or rectangular or irregular in shape due to presence of stomata. Vascular bundles are collateral having xylem and phloem. The transverse section of *Emex spinosa* stem revealed that epidermial cells were closely packed and rectangular in shape, about 15-25µm in length and 7-9µm in width. Epidermis is followed by cortex having 18-32µm length and 8-16µm width. The inner layer of the cortex is endodermal lining which are closely packed barrel shaped cells 18-22µm in length and 12-16µm in width. The vascular bundles were collateral, open and arranged in a ring. Phloem length 18-22µm and width 22-33µm, xylem length 19-33µm and width 22-28µm. Pith is absent in *Emex spinosa* stem.

Organoleptic and phytochemical analysis

Organoleptic evaluation showed that stem has bitter taste and indistinct odor (Table No. 1). The phytochemical screening of leaf and root showed Alkaloids, Flavonoids, Phytosterols, Proteins, Phenols and Triterpenoids, while Carbohydrates and Glucosides were present in leaf only (Table No. 3). Total phenolic content of stem was 77.84 mg/g with standard deviation of ±0.32 which is relatively less than the total phenolic content of fruit i.e. 97.09 mg/g with standard deviation ±0.18 and leaf which is 97.7 mg/g with standard deviation value ±0.26 (Table No. 4). Total flavonoid content of stem was 15.08 mg/g

standard deviation ±0.28, which is relatively lower than the fruit i.e. 37.39 mg/g with standard deviation value 0.12. Leaf had high content of flavonoid i.e. 58.66 mg/g and standard deviation ±0.38(Table 4). DPPH Radical Scavenging Assay Fruit showed comparatively highest % of DPPH radical scavenging activity i.e. 90.45 % and SD ±0.26 followed by leaf i.e. 79.32% and SD ±0.22 and lower DPPH radical scavenging effect by stem which was 48.33% and SD ±0.16 (Table 4). Total antioxidant capacity showed by stem was 50.74, leaf 25.37 and fruit 42.01 (Table 4).

Table 1. Morphological and Organoleptic evaluation.

Plant parts	Features	Fresh	Dry
Root	Odor	Indistinct	Indistinct
	Color	Dull brown	Dark brown
	Shape	Cylindrical	Cylindrical
	Rootlets	Present	Present
	Direction of growth	Vertical downward	Vertical downward
	Fracture	Fibrous	Brittle
	Texture	Smooth	Smooth
	Taste	Bitter	Bitter
Stem	Color	Light green	Dark green
	Odour	Indistinct	Indistinct
	Shape	Cylindrical	Cylindrical
	Phyllotaxis	Spiral	Spiral
	Kind	Herbaceous	Herbaceous
	Direction of growth	Upright	Upright
	Fracture	Soft	Brittle
	Texture	Smooth	Smooth
	Taste	Slightly bitter	Indistinct
	Dimension	Length= 13-17cm; Width= 1.5-2.5cm	.....
Leaf	Dimension of leaflet	Length=3-5cm; Width= 1.5-2cm	.....
	Color	Upper epidermis dark green; lower light green	Both surfaces dark green
	Incisions	Deep incision	Deep incision
	Composition	Compound	Compound
	Venation	Reticulate unicostate	Reticulate unicostate
	Margin	Spiny dentate	Spiny dentate
	Apex	Acute	Acute
	Leaf shape	Lanceolate	Lanceolate
Flower	Odor	Indistinct	Indistinct
	Taste	Slightly bitter	Slightly bitter
Fruit	Size	2-2.5mm length: 1-1.5mm width	2-2.5mm length: 1-1.5mm
	Kind	Achene	Achene
	Dimension	3-4.5mm long, 2-3mm broad	3-4.5mm long, 2-3mm
	Shape	Obovoid	Obovoid
	Dehiscence	Irregular	Irregular
	Placentation	Basal	Basal
	Odour	Indistinct	.....
	Taste	Bitter	Bitter

Table 2. Stomatal Study of *Emex spinosa* leaf.

Epidermis	Type of Stomata	Stomatal Index	Size of stomata pore		Size of guard cells	
			L(µ)	W(µ)	L(µ)	W(µ)
Upper	Hemiparacitic	24.34 ± 0.60	15	9	26	18
Lower	Anisocytic Anomocytic	35.04 ± 0.59	25	12	28	16

Table 3. Phytochemical Screening of *Emex spinosa*.

Parts Used	Phyto-constituents					
	Carbohydrates	Proteins	Triterpenoids	Alkaloids	Flavenoids	Glycosides
Leaf	+	+	+	+	+	+
Fruit	_	_	_	+	+	_
Stem	_	+	+	+	+	_

Table 4. Analysis/ tests of various activities of *Emex spinosa*.

Analysis / Test	Part used	Total Phenolic Content (mg/g) (a, b)*	Total Flavonoid Content (mg/g) (a, b)	DPPH Radical Scavenging Assay (%) (a, b)	Total Antioxidant Capacity	
Chemical Analysis	Stem	77.84± 0.32	15.08±0.28	48.33±0.16	50.74	
	Leaf	97.09±0.26	58.66±0.38	79.32±0.22	25.37	
	Fruit	97.72±0.18	37.39±0.12	90.45±0.26	42.01	
Acute toxicity test		Extract Group	Dose (mg/kg)	Total no of tested mice	Mortality after 4 hours	
		Control (saline)	10mg	6	0	
		Methanolic extract	50mg	6	0	
		Methanolic extract	100 mg	6	5	
		Methanolic extract	150mg	6	6	
Phytotoxic Activity	Part used	Extracts	Dose (µg/ml)	No of fronds in test	No. of fronds in control (- ive)	% inhibition
	Stem	Methanolic	10	28	39	28.2
			100	25		35.8
			1000	23		41.0
	Leaf	Methanolic	10	25	39	35.8
			100	23		41.0
			1000	21		46.1
	Fruit	Methanolic	10	26	39	33.3
			100	25		35.8
			1000	18		53.8
Antispasmodic Activity	PART USED	GROUP	DOSE /KG	% Intestinal transit	% Inhibition	
		Normal	-----	73.30 ± 1.60	------	
		Control	------	81.33 ± 2.13	-------	
	Stem	Methanolic extract	10*mg*	65.65± 1.76	10.0	
		Methanolic extract	20 mg	70.10±*1.41*	4.3	
		Methanolic extract	30mg	69.00±2.55	5.8	
	Leaf	Methanolic extract	10mg	61.63±2.30	15.9	
			20mg	70.70±3.00	3.5	
			30mg	70.56±1.37	3.7	
	Fruit	Methanolic extract	10mg	55.40±2.07	24.4	
			20mg	64.32±1.73	12.3	
			30mg	63.76±1.68	13.0	
		Atropine sulphate	5 mg	32.29 ± 1.02	55.9	
Cytotoxic Activity	Part used	Extracts	Extract Conc. (µg/ml)	Total No. of Larvae	No. of survivors	No. of dead shrimps	Percent mortality	LD_{50}
		Control	0	30	30	0	0	
	Stem	Methanolic	5	30	24	6	20	525
			50	30	20	10	33.3	
			500	30	19	14	46.6	
	Leaf	Methanolic	5	30	20	10	33.3	20
			50	30	8	22	73.3	
			500	30	6	24	80	
	Fruit	Methanolic	5	30	23	7	23.3	47
			50	30	13	17	56.6	
			500	30	10	20	66.6	

1. Mean Values Obtained From Experiments Performed in Triplicate
2. Mean Value Determined Graphically and Standard Deviation
3. Values are Mean, ± Standard Error of Mean
4. Each Value Represents Average of Six Determinations.

Pharmacognostic and pharmacological studies

Methanolic extract of *Emex spinosa* showed toxicity at concentration of 100mg and 150 mg. Total tested 6 mice were tested, 5 mice were dead (90%) after 4 hours while 100% mortality was recorded at 150 mg. Whereas at 50mg the extract showed no toxic effect. Similarly no death was recorded at control (Table No. 4). Results of in vitro showed that the stem methanolic extract of *Emex spinosa* at 50µg/ml and 500µg/ml has significant effect as compared to 5µg/ml (Table No. 4). The percent lethality at 5µg/ml, 50µg/ml and 500µg/ml was 20%, 33.3% and 46.6% respectively and LD50 value 52µg/ml. Leaf methanolic extract showed 33.3%, 73.3% and 80% lethality at 5µg/ml, 10µg/ml and 500µg/ml respectively and LD50 value of 20 µg/ml (Table No. 4). Fruit methanolic extract showed 23.3%, 56.6% and 66.6% lethality at 5µg/ml, 10µg/ml and 500µg/ml respectively, and LD50 value of 47µg/ml (Table No. 4). The phytotoxic activity of leaf methanolic extract of dose 1000µg/ml has more inhibition as compared to 10µg/ml and 100µg/ml i.e. 41% and 35.8% and 28.2% at 100 and 1000µg/ml (Table No. 4). The leaf methanolic extract showed phytotoxicity at 1000µg/ml having a percentage inhibition of 46.1% while at 100µg/ml and 10µg/ml inhibition was 41.0% and 35.8% respectively with (Table No. 4). The fruit methanolic extract showed phytotoxicity at 1000µg/ml having 53.8% inhibition, while 100µg/ml and 10µg/ml resulted 35.8.0% and 33.3% inhibition respectively (Table No. 4). The effects of the methanolic extracts of different doses of stem, leaf and fruit of *Emex spinosa* revealed that the stem methanolic extract at a dose of 10mg/ml have significant effect i.e. 10% inhibition as compared to 20mg/ml and 30mg/ml which have 4.3% and 5.8% inhibition respectively (Table No. 4). The leaf methanolic extract showed a remarkable antispasmodic effect at 10mg/ml having percent inhibition of 15.9%, while at 20mg/ml and 30mg/ml caused 3.5% and 3.7% inhibition respectively (Table No. 4). The fruit methanolic extract showed significant intestinal transit inhibition at all doses but maximum at 10mg/ml i.e. 24.4% while at 100mg/ml and 10mg/ml 12.3% and 13% inhibition respectively (Table No. 7).

Discussion

Morphological and anatomical characteristics

Plants are source of phyto-constituents showing different pharmacological property, isolating such impending plants is of importance in medicine (Padmavathy, 2010). The transverse section of *Emex spinosa* stem showed three different regions from outside to inside. The transverse section of the leaf shows epidermis, Mesophyll parenchyma tissue and vascular bundles. In *Emex spinosa* the stomata are paracytic type and present on both surface (amphistomatic) of leaf stomata index in upper is 24.34±0.60, and in lower side 35.04±0.59.

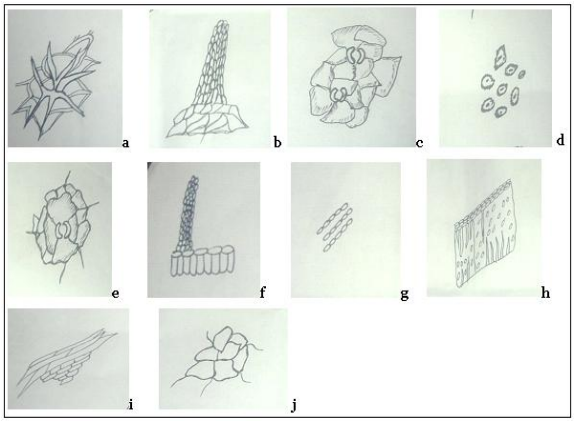

Fig. 1. Powder drug of *Emex spinosa* Leaf.

Kay: a) Star shaped trichome b) multicellulartrichome c) stomata with epidermal cells d) starch grains e) anisocytic stomata f) epidermal cells with palisade cells and multicellular trichome g) fragments of epidermal cells h) vessels attached with paranchymatous cells i) fibers from vein with paranchymatous cells j) spongy mesophyll cells.

Pharmacognostic and pharmacological studies

Recently several workers studied the pharmacognostical values in leaves of *Cordia rothii* (Ghori, 2011) and *Glycosmis pentaphylla* (Arora, 2011); stem bark of *Ficus racemosa* (Ahmed, 2011); stem bark and leaf of *Termina liacitrine* (Ingle, 2011); roots and leaves of *Blepharis molluginifolia* (Patta, 2011) and leaves, stem, roots of Macrotyloma suniflorum (Kumar, 2011). Despite the modern methods, identification and evaluation of plants drug by pharmacognostical studies is still more accurate,

reliable and inexpensive (Ahmed, 2011). The phytochemical investigation revealed the presence of carbohydrates, proteins, phenols, alkaloids, triterpenoids and flavonoids. The same result was also confirmed by Rizk (1986). The acute toxicity test on mice displayed no mortality or behavior changes at 50 mg/kg and so the crude extract was considered to be safe up to this dose. The drug of *Emex spinosa* could be safe up to the dose of 500mg/kg (Raheim *et al.,* 2014); the possible reason for the change results might be the administration of the drugs. For free radical scavenging activity against 2,2-diphenyl-2-picrylhydrazyl (DPPH), highest value was shown by fruit extract i.e. 90.45% and lowest by stem 48.33%. Aqueous leaves ethanol extract (70%) of *Emex spinosa* displayed free radical scavenging action in response to 2,2- diphenyl-2-picrylhydrazyl (DPPH), Rutin and luteolin isolated from *Emex spinosa*, showed significant scavenging activity (Emam *et al*., 2010). More potent radical scavenging effect is obtained by the greater concentration of phenolic compounds 97.72 mg/g and flavonoids 37.39mg/g highest in fruit (Pourmurad *et al*., 2006). Large amounts of phenolic compounds may provide antioxidant properties (Shankar *et al.,* 2008). In cytotoxic activity the order of toxicity for brine shrimps was leaf > fruit > stem in all the three concentrations (5, 50, 500µg/ml). Stem methanolic extract did not show any significant cytotoxic activity. However, leaf exhibited lower LD50 values 20 ug/ml as compared to LD50 value of the stem (525ug/ml). Leaf extract could be the potential one which might possess potential cytotoxic compounds. According to Raheim *et al*., (2014), Aloe Emodin glucosides extracted from *Emex spinosa* showed anticancer activity against HCT, Caco-2, HepG-2 and MCF-7. The extracts with LC 50 value higher than 200 mg/l in the brine shrimp test can be considered inactive (Anderson *et al*., 1991). The phytotoxicity obtained from the crude methanolic extracts of *Emex spinosa* was carried out against *Lemna minor*. Stem and leaf extracts did not show any considerable activity at even highest doses (41% and 46%) respectively, while fruit extract showed moderate activity at the highest dose 1000 µg/ml (53%). Contradicting to the investigation of Inderjit and Duke (2003) that the toxins produced by *Emex spinosa* might reduce the chlorophyll contents of the susceptible plant which leads to the reduction of plant growth. *Emex spinosa* contained some allelo-chemicals which has arrested the growth of test species. Studies suggested that *E. spinosa* is an allopathic plant which reduces the growth and germination of different test species (Naseem, 2013). These results obtained above from laboratory tests suggest that *E. spinosa* possess allelophatic substances. Field experiment must be conducted to examine the effectiveness under natural condition. Antispasmodic activity displayed that when fruit methanolic extract was administered at the dose of 10mg/kg significantly inhibited (24%) gastro intestinal tract (GIT) mortality followed by leaf extract (15%) and lowest inhibition was shown by stem (4.3%) as compared to Atrophine sulphate (55.9%). It has been reported that morphine and related drugs i.e. Atropine sulphate inhibits gastrointestinal mortality by acting at spinal, superaspinal and peripheral receptor (Galligan *et al.,* 1983). In conclusion *Emex spinosa* methanolic extract cause anti GIT motality and it may be due to the presence of flavonoids and triterpenoids (Subhan *et al.,* 2010).

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