BIOLOGICAL AND PHARMACOLOGICAL STUDIES OF *HELIOTROPIUM DASYCARPUM* LEDEB

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خلاصہ

اس مطالعہ میں، ہیلیوٹروپیمیم داسیاسی کارپم *Heliotropium dasycarpum* کے میتانولک نکاسی نے اس کی تشخیص کی تھی کہ اس کی حیاتیاتی اور فارماسولوجیاتی سرگرمیاں. اینٹی بیکٹیریل کے لئے، اینٹی فنگل اور فیوٹوٹوکسیکی سرگرمیاں 11 بیکٹیریا للیل، 10 فنگل اور 4 پودے لگانے والی پرجاتیوں کو باقاعدگی سے استعمال کیا گیا تھا. پلانٹ نے صرف 3 بایکٹیریل (چلیمیڈیاتراچومیٹس، β-ہامولوٹک اسکرپٹوکیسی اور کیوری نیبیکٹیریم ڈفیریایا) اور چار فنگل پرجاتیوں (نیوموکیکیسس جروویسی، کرپٹوکوکس نروپرفارٹس، زیوگومیکوسس نروپرفارمز، زیوگومیکوس اور مائیکروسافم کینس) کے مقابلے میں زیادہ سے زیادہ غیر معمولی حراستی کے خلاف واقعہ علاقے کو دکھایا. 60، 120 اور 240 ملی گرام / ایل ایل اور کوئی انعقاد زون 10، 20 اور 30 ملیگرام / ملی میٹر کی کم سے کم روک تھام حراستی (مائیک) کے ساتھ ذکر کیا گیا تھا. Phytochemical تحقیقات پلانٹ alkaloids، cardenolides، saponnins، flavonoids، انزائموں، terpenoids اور سورج کی واقعہ پر مبنی ہے. نتائج نے یہ بھی اشارہ کیا کہ ایچ ڈیسی کارپم میں اہم فیوٹوٹوکسیک سرگرمیاں موجود تھیں. پلانٹ نامزد کردہ زیادہ سے زیادہ انڈونیشن لیمانابالغ ایل (100٪) اور ایچھرنیایاکراسپس (مارٹ.) سلمز-لاؤب کی ترقی کے لئے. (75٪) اور کم از کم نمائش کاسلولوس آرویسیس ایل (37٪) اور ایلیوس ریٹینس (ایل) گاوڈ (20٪) کے لئے ذکر کیا گیا تھا.

Abstract

In this study, methanolic extract of *Heliotropium dasycarpum* was assessed to explored for its biological and pharmacological activities. For antibacterial, antifungal and phytotoxicity activities 11bacterial, 10 fungal and 4 weed plant species were used respectively. Plant showed inhibition zone against only 3 Bacterial (*Chlamydia trachomatis*, *β-hemolytic streptococci* and *Corynebacterium diphtheria*) and four fungal species (*Pneumocystis jiroveci*, *Cryptococcus neoformans*, *Zygomycetes* and *Microsporum canis*) at maximum inhibitory concentration like; 60, 120 and 240 mg/ml and no inhibition zone was noted with minimum inhibitory concentration (MIC) of 10, 20 and 30 mg/ml. Phytochemical investigation exposed the occurrence of plant alkaloids, cardenolides, saponnins, flavonoids, enzymes, terpenoids and pigments. Results also indicated that *H. dasycarpum* had important phytotoxic activities. Plant designated maximum inhibition for the growth of *Lemna minor* L. (100 %) and *Eichhornia crassipes* (Mart.) Solms-laub. Maximun (75%) and minimum (20 % and 37 %) inhibition was noted for *Elymus repens* (L.) Gould and *Convolvulus arvensis* L. respectively.

Introduction

The study of medicines or crude drugs produced from natural sources such as plants, microbes, and animals is known as Pharmacognosy. It includes analysis of their biological, chemical, biochemical, and physical properties. Since the beginning of human civilization, plants have been used as a source to cure different illnesses of humans and animal. Different researchers have documented various plants of medicinal value in nook and corner of the world (Akhtar *et al.,* 2000; Baloch *et al.,* 2016). More than 75% people around the world depend on these medicinal plants for the production of different types of medicines (Ahmad *et al.,* 2007). Recent studies showed that medicinal plants are also a great source of natural compounds which act as anti-infection and play a vital role in the field of ethno-pharmacology (Rios and Racio, 2005). Provision of basic health care facilities depends on the availability of suitable medicines. Plants are being successfully used as an important source of various medicines to treat different kinds of illness. Some of them are used as standard preparations and some are used as a source for obtaining the extracts to make different formulations. The extensive research on the plant extracts has enabled the scientists to identify a variety of compounds which possess medicinal value (Farnsworth *et al.,* 1985). In the 19th century, due to the advancements in the field of pharmaceutical chemistry especially the medicinal chemistry more than 25% of drugs used in well developed

countries are of plant origin and about 120 plant derived substances are used in modern system of medicines worldwide (Sharma *et al.,* 2009). Mazhar *et al.,* (2015) reported that in plant several elements are existing, these have many means of act which includes more or less proteins, enzymes and metabolites, including vitamin, pigments, flavonoids and additional components of phenol these components are the foundation of antimicrobial and antioxidants power of any plant products. Drug researchers often make use of ethno-botany to hunt for the naturally occurring pharmacologically active substances. Many of the common drugs such as opium, digoxin, aspirin and quinine are obtained from botanic sources. The therapeutic efficacy of a medicinal product depends on the quality of medicinal plant (Joharchi *et al.,* 2012). So the medicinal plants are widely being used as standardized phyto-medicines having high quality, with effective results (Calixto, 2005). Like many other developing countries in Pakistan, a high percentage of the population depends on traditional medicine for primary health care (Tareen *et al.,* 2011). Pakistan has a diverse range of climatic and phytogeographic conditions which results in diverse flora containing several medicinal plant species. Estimated total flora of Pakistan is comprised of 6000 species (Shinwari *et al.,* 2000). Very small number of these plants are analyzed for their photochemical properties and still there are large number of plants need their photochemical analysis.

The main objective of this research was to analyze the presence or absence of different phytochemicals, biological and pharmacological activities of *H. dasycarpum*. As there is no data available on the *H. dasycarpum.* The current study is designed to fill up this gap and to help in the standardization of the drug used in traditional system of medicines.

Materials and Methods

Plant used for investigation: *H. desycarpum* is an important medicinal plant belonging to the angiosperm family Boraginaceae. This species grows as a perennial plant in arid and semi-arid habitats of Pakistan, Iran, Afghanistan and central Asia (Khatamsaz, 1991). In Balochistan the province of Pakistan, *H. desycarpum* is distributed in Mastung, Kalat, Zehri, and Neemargh areas and locally known as Sagdaroo and known as medicinal herb (Baloch *et al.,* 2016). Its extract is used by local folks for the cure of eye infection (Tareen *et al.,* 2010). The plant is also reported to possess antimicrobial and phytotoxic effects (Ghaffari *et al.,* 2013).

Plant Material Collection, Sample Preparation and Extraction Process: Fresh whole plant (*H. dasycarpum*) material was collected from natural habitat of plant after proper identification. The plant leaves were eroded away wisely with purified water and then material was dried for 10-12 days at room temperature. Dry plant materials were powdered by electric blender and methanol solvents was added. For extraction processes, the procedure used by Dupont *et al.,* (2006) was accepted by slight alteration. Temporarily, 25 gm ground plant materials were individually weighed and saturated with 125ml methanol on maximum temperature for 75h in steady shaking condition. Then extract was sieved by the use of No.1filter paper. For dryness the filtrate was evaporated on 35^{o}C temperature in evaporating dish. Yield percentage was determined through following formula;

$$\textbf{Yield percentage} = \frac{\textbf{Amount of dried extract (g)}}{\textbf{Amount of powered plant sample (g)}} \times \textbf{100}$$

Preliminary Phytochemical Tests: For chemical screening following testes were perform on *H. dasycarpum.* Alkaloids was examined by using the Wagner and Draggendoff elements as adopted by Sofowora, (1994). Blood haemolysis method was utilized to examine the presence of Saponnins (Sofowora, 1994). Borntrager procedure was used for the examination of combined anthraquinones as the method described by De *et al.,* (2010). The occurrence/presence of cardenolides was detected by keller-Kiliani method as used by Srivastava *et al.,* (1991). The appearance of deep green color indicate the occurrence of tannins (Trease and Evans, 1989). All other phytochemicals (Polyphenols, Pigments, Carotenoids, Terpenoids and Enzymes) identification test was carried out by Thin-layer chromatography (TLC) and the confirmation of the presence of different functional groups was carried out through FT-IR spectroscopic analysis in Eastern Medicine Department University of Balochistan Quetta.

Antibacterial Assay; The methanol extracts of the plant material were examined for 11 antibacterial species (*Eschericha coli, Klebsiella* spp. *Shigella flexinari, Bacillus subtilis, Staphylococcus aureus, Chlamydia trachomatis, Pseudomonas aeruginosa*, β-hemolytic streptococci, *Corynebacterium diphtheria*, *Proteus mirabilis* and *Salmonella typhi)* by using agar medium. Each experiment was done in triplicates as the method used by Osungunna and Adedeji, (2011).

Antifungal Assay: Standard way out of methanolic extract of *H. dasycarpum* was tested against 10 different fungal species (*Coccidioides*, *Blastomyces dermatitidis*, *Pneumocystis jiroveci*, *Cryptococcus neoformans, Zygomycetes, Fusarium solani*, *Microsporum canis, Aspergillus flavus, Candida albicans* and *Candida glabrata*). Paper disc diffusion method was followed for antifungal movement investigation (Kumara *et al.,* 2009).

Minimum Inhibitory Concentration: Agar dilution method was used for minimum inhibitory concentration (MIC) assay. Different concentrated (10, 20, 30, 60, 120 and 240mg/ml) plant extracts were developed through the dilution of serial and then permitted to set in the plates.

Phytotoxicity Assay: Four different weed plants including, *Elymus repens* (L.) Gould, *Lemna minor* L., *Convolvulus arvensis* L. and *Eichhornia crassipes* (Mart.) were used to test the phytotoxicity of *H. dasycarpum*. For phytoxicity assay e-medium was developed and its pH was upheld within 5.5 - 6.0 with the adding KOH pellets. Eight groups of 20 bottles (each for 500, 50, 5ppm and control) were equipped for examination following Rahman *et al.,* (2001) method.

Results and Discussions

Yield Extraction:

$$\textbf{Yield percentage} = \frac{\textbf{Amount of dried extract (g)}}{\textbf{Amount of powered plant sample (g)}} \times \textbf{100}$$

$$= \frac{1.5}{25} \times 100$$

$$= 6\ \%^{\text{ w/w}}$$

The calculated extraction yield discloses the yield percentage to be 6% w/w.

Phytochemical Properties: Results showed the presences of Alkaloid, Saponnins, Cardenolides, flavonoids, polyphenols, pigments, carotenoids, terpenoids and Enzymes. However Tannins, free anthraquinons and bound anthraquinone were absent in the *H. dasycarpum* methanol extract (Table 1). The presence of alkaloids and other chemicals in *H. dasycarpum* are argument with the preceding investigation that were completed in many plants of *Heliotropium* genus (Ghaffari *et al.,* 2013). However the presences of Saponins in *H. dasycarpum* in current observation is contradict to the results of Ghaffari *et al.,* (2013). The saponins of *H. dasycarpum* are deliberated to be the feature control for biological activity of products extracted from this plant. This movement be governed through the level and the configuration of vigorous saponins, which reflect the prejudiced by the topographical origin of plant material (Dinchev *et al.,* 2008). In nature the species of *Heliotropium* are very poisonous because of the occurrence of pyrrolizidine alkaloids as main component that might be the cause of human deaths owing to unintentional ingesting of these plants in diverse area of the world. The pyrrole metabolites were designed by pyrrolizidine alkaloids through liver microsomal oxidation that resulted liver injury to the effected persons (Tandon *et al.,* 1978). Consequently the upstairs positive consequence of alkaloid test indicated the toxicity of the plant to the other plants, human and animal's population.

Antibacterial Activity: Antibacterial screening exhibited that the *H. dasycarpum* methaanolic extract was effective against only 3 (*β-hemolytic streptococci, Chlamydia trachomatis and Corynebacterium diphtheria),* bacteria out of 11 investigated bacterial species, at diverse concentrations and plant extract was ineffective against other 9 tested bacteria at all the concentrations extract (Table 2.) However *Chlamydia trachomatis* and *Corynebacterium diphtheria* replied absolutely through showing inhibition zones (6, 9 mm and 7, 10 mm) to two of six concentration verified, that was 120 and 240 mg/ml respectively. Other one species β-*hemolytic streptococci* responded by having zone of inhibition (4, 8 and 11 mm) to three of six concentrations that was, 60, 120 and 240 mg/ml respectively. The positive response of H. *dasycarpum* extract can be accredited to the occurrence of different secondary metabolites and saponnins and its contrary to bacterial activity has been already recognized by other researchers including Tschesche, (1971), Ghaffari *et al.,* (2013), Osungunna and Adedeji, (2011). Scott and Osho, (2012) and Adegoke, (1968) observed and indicated various secondary metabolites particularly pyrrolizidine alkaloids, saponins, tannins and triterpenoids were established to be accountable for the antimicrobial activity in the species of genus *Heliotropium*. Also, the heavy-duty extraction volume of methanolic might have formed better quantity of dynamic contents accountable for activity against bacteria. In this study plant extract concentration in between 10, 20 & 30mg/ml showed non inhibitory activity through MIC of all examined bacterial species. It is analogous to the action of the standard utilized at 10mg/ml concentration, which showed activity on all the bacterial species of this investigation. Many other researchers (Murray *et al.,* 2009; Ullah *et al.,* 2009; Angeh *et al.,* 2007) reported that one possible basis for new

antimicrobials might be a plants. Commonly aromatic imitative entitled filifolinol got from *Heliotropium* species presented anti-microbial action for the reason that these plants had aptitude to grow in extreme environmental conditions. Thus these constituents has important part in the protection mechanism of the any plant (Urzua *et al.,* 2008; Torres *et al.,* 1994; Modak *et al.,* 2007).

In this study, antibacterial activity of *H. dasycarpum* was found very poor. The inhibition zone showed against only three bacterial species by methanol plant extract was very small as compared to the standard drug named Imipenem and against all other (9) bacterial species there found no zone of inhibitions in all concentration (Table 2). Similar statement was also described by Ghaffari *et al.,* (2013). They found no antimicrobial activity against 6 different tested organisms (*Eschericha coli, Bacillus subtilis, Shigella flexinari, Staphylococcus aureus, Pseudomonas aeruginosa and Salmonella typhi*).

Antifungal Activity: In *H. dasycarpum* antifungal activity was carried out against 10 different fungal species (*Coccidioides, Blastomyces dermatitidis, Pneumocystis jiroveci, Cryptococcus neoformans, Zygomycetes, Fusarium solani, Microsporum canis, Aspergillus flavus, Candida albicans & Candida glabrata)* and the results exposed through methanol sections of plants are illustrated in Table 3. The methanolic extraction of *H. dasycarpum* displayed 4, 6 & 10 mm inhibition against *Pneumocystis jiroveci* in different concentration of plant extract; 60, 120 and 240 mg/ml respectively. 8, 12 mm inhibition zone was found against *Cryptococcus neoformans* in 120 and 240mg/ml plant extract concentration. *H. dasycarpum* showed 14, 16 and 20 mm inhibition zone against *Zygomycetes* and 27, 30 and 32 mm zone against *Microsporum canis* in 60, 120 and 240 mg/ml concentration of plant extract, while *H. dasycarpum* fraction was inactive against other remaining tested 6 fungal species. Similar observation was also reported by Ghaffari *et al.,* (2013), they found 25% inhibition zone against *Microsporum canis* in methanol extract of *H. dasycarpum.* The consequences of antifungal activities of the plant are in consistent with other plant species like; *Echium rauwolfii, Echium horridum* (El-shazly *et al.,* 1999), *Cordia curassavica* (Ioset *et al.,* 2000), *Cordia linnaei* (Ioset *et al.,* 1998), *Cordia morelosana* (Sanchez *et al.,* 2009), *Arnebia euchroma* (Damianakos *et al.,* 2012), *Arnebia hispidissima* (Shukla *et al.,* 1969), *Trichodesma amplexicaule* (Singh and Singh, 2003) and *Cynoglossum officinale* (Plyta *et al.,* 1998) of family Boraginaceae. Ahmad *et al.,* (2009) also reported considerable activities through crude methanolic fraction of *Onosma griffiithii* against *Fusarium solani* and *Aspergillus flavus*, whereas its ethyl acetate and n-butanol extraction showed inactivity. Similarly non antifungal activities were documented when ethanolic and aqueous extract of the plant; *Colendia procumbents* was examined in contradiction of *Candida albicans* (Ramakrishnan *et al.,* 2011). Bahraminejad, (2012) also reported no activity in methanolic and aqueous extraction of *Anchusa italic* and *Trichodesma zeylanicum.*

Phytotoxic Activity: The data regarding phytotoxic activity (Table 4) of metanolic extract of *H. dasycarpum* against 4 different weeds plants (*Elymus repens* (L.) Gould, *Lemna minor* L., *Convolvulus arvensis* L. and *Eichhornia crassipes* (Mart.) Solms-laub.) are shown in Table 4. Consequences showed that methanol extract of plant showed 20%, 100%, 37% & 75% phytotoxic efficiency against *E. repens, L. minor, C. arvensis* and *E. crassipes* on 1000µg/ml concentration respectively. However in 100µg/ml inhibition % decrease by 5%, 72%, 8% and 28% and at 10 µg/ml concentration inhibition was recorded 0 %, 28%, 0 % and 13% for *E. repens, L. minor, C. arvensis* and *E. crassipes*, respectively. Observation reported by Ghaffari *et al.,* (2013) are in agreement with our results as they found 100 %, 65 % and 25 % phytotoxic activity against *L. minor* at different concentration such as 1000µg/ml, 100µg/ml and 10µg/ml of *H. dasycarpum* methanol extract, respectively. The variation in inhibition rate against *L. minor* at different concentration might be due to the topographical origin of plant material (Dinchev *et al.,* 2008). In this study highest activity showed by 1000 µg/ml and lowest at 10 µg/ml concentration. Previously study on phytotoxic measurement in *Helitropium* species particularly aqueous extract of *H. indicum* displayed allelopathic growth parameter on wheat rootlet (Mongelli *et al.,* 1997). Shah *et al.,* (2015) studied the phytotoxic effect of *Heliotropium strigosum* ethyl acetate extract and found maximum inhibition % age maximum concentration (1000 µg/ml). The species of *Heliotropium* genus had valued propensity to the plant-toxicity nonetheless more research is obligatory to be discussable.

Table 1: Phyto-chemical properties of methanolic extract of *Haliotropium dasycarpum* Ledeb.

Phytochemicals	Present/Absent (+/-)	Phytochemicals	Present/Absents (+/-)
Alkaloid	+	Tannins	-
Cardenolides	+	Polyphenols	+
Free anthraquinone	-	Pigments	+
Bound anthraquinone	-	Carotenoids	+
Saponnins	+	Terpenoids	+
Flavonoids	+	Enzymes	+

Table 2: Anti-bacterial efficacy of methanolic extract of *H. dasycarpum*

Bacterial Species	**Average diameter of inhibition zone (mm)**						**Control drug (mg/ml)**
	Concentration of plant extract (mg/ml)						
	10	20	30	60	120	240	10
Escherichia coli	-	-	-	-	-	-	26
Klebsiella spp.	-	-	-	-	-	-	27
Shigella flexinari	-	-	-	-	-	-	25
Staphylococcus aureus	-	-	-	-	-	-	50
Bacillus subtilis	-	-	-	-	-	-	50
Chlamydia trachomatis	-	-	-	-	6	9	22.4
Pseudomonas aeruginosa	-	-	-	-	-	-	19.5
β-hemolytic streptococci	-	-	-	4	8	11	20
Corynebacterium diphtheriae.	-	-	-	-	7	10	23
Proteus mirabilis	-	-	-	-	-	-	20
Salmonella typhi	-	-	-	-	-	-	25

Diameter of cork borer = 6 mm

Table 3: Antifungal efficacy of the methanol extract of *H. dasycarpum*

Fungal Species	Average diameter of inhibition zone (mm)						Control drug (mg/ml)	Standard Drug used for control
	Concentration of plant extract (mg/ml)							
	10	20	30	60	120	240	10	
Coccidioides	-	-	-	-	-	-	80	Amphotericin B
Blastomyces dermatitidis	-	-	-	-	-	-	70	Amphotericin B
Pneumocystis jiroveci	-	-	-	4	6	10	100	Corticosteroids
Cryptococcus neoformans	-	-	-	-	8	12	100	Histoplasma
Zygomycetes	-	-	-	14	16	20	98.5	Histoplasma
Fusarium solani	-	-	-	-	-	-	89.6	Miconazole
Microsporum canis	-	-	-	27	30	32	99	Miconazole
Aspergillus flavus	-	-	-	-	-	-	100	Amphotericin B
Candida albicans	-	-	-	-	-	-	100	Miconazole
Candida glabrata	-	-	-	-	-	-	87	Miconazole

Diameter of cork borer = 6 mm.

Table 4: In vitro phytotoxic activities of *H. dasycarpum*

Plant Name	Conc. Of Compound (µg/ml)	No. of Fronds		% Growth Regulation	Standard Drug *(Paraquat)* Concentration (µg/ml)
		Sample	**Control**		
Elymus repens (L.) Gould	1000	0	20	20	0.015
	100	10		5	
	10	20		0	
Lemna minor L.	1000	0	26	100	
	100	10		72	
	10	20		28	
Convolvulus arvensis L.	1000	0	22	37	
	100	10		8	
	10	20		0	
Eichhornia crassipes (Mart.) Solms-laub.	1000	0	20	75	
	100	10		28	
	10	20		13	

Conclusion and Recommendation

So, the conclusion of study is that investigated plant species can be served as a valuable obstinacy for the handling of poisons caused by only these three bacteria like; *β-hemolytic streptococci, Chlamydia trachomatis and Corynebacterium diphtheria.* However, efficacy absorbed examine is essential for this plant species by a vision to segregating and depicting the dynamic metabolites which are responsible for the detected activities. Further that the upstairs literature and new work delivers technical foundation for the utilization of the plant *H. dasycarpum* as a strong herbicidal manager that delivers new eon of separation of its phytochemical constituents. The crop might grow in hard conditions of the environment so low antifungal and antibacterial activities must be occupied under deliberation for the future investigation.

References

Adegoke, A.L. (1968). West African plants folkore research. *J. Ethnopharmacol*. 5: 145-50.

Ahmad, B., Ali, N., Bashir, S., Choudhary, M.I., Azam, S. and Khan, I. (2009). Parasiticidal, antifungal and antibacterial activities of Onosma griffithii Vatke. *African Journal of Biotechnology*. 8: 5084-87.

Ahmad, B., Shah, S.H., Bashir. S., Nisar, M. and Chaudry, M.I. (2007). Antibacterial and antifungal activities of Andrachne cordifolia Muell. *Journal of enzyme inhibition and medicinal chemistry*. 22: 726-9.

Akhtar, M.S., Iqbal, Z., Khan, M.N. and 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: 99-107.

Angeh, J.E., Huang, X., Sattler, I., Swan, G.E., Dahse, H., Härtl, A. and Eloff, J.N. (2007). Antimicrobial and anti-inflammatory activity of four known and one new triterpenoid from *Combretum imberbe* (Combretaceae). *Journal of ethno-pharmacology*. 110: 56-60.

Damianakos, H., Kretschmer, N., Sykłowska-Baranek, K., Pietrosiuk, A., Bauer, R. and Chinou, I. (2012). Antimicrobial and cytotoxic *isohexenylna phthazarins* from *Arnebia euchroma* (Royle) Jonst.(Boraginaceae) callus and cell suspension culture. *Molecules*. 17: 14310-22.

De, S., Dey, Y. N. and Ghosh, A. K. (2010). Phytochemical investigation and chromatographic evaluation of the different extracts of tuber of *Amorphaphallus paeoniifolius* (araceae). *International Journal on Pharmaceutical and Biomedical Research*. 1(5), 2010, 150-157.

Bahraminejad, S. (2012). In vitro and in vivo antifungal activities of Iranian plant species against Pythium aphanidermatum. *Ann Biol Res*. 3: 2134-43.

Baloch, A.H., Baloch, I.A., Haneef–ur-Rehman, S.R.S. and Jaffar A. (2016). Contribution to the Knowledge of Ethnobotany of Balochistan, Pakistan. *Lasb U J Sci Techl*. 1: 143-73.

Calixto, J.B. (2005). Twenty-five years of research on medicinal plants in Latin America: a personal view. *Journal of ethnopharmacology*. 100: 131-4.

Dinchev, D., Janda, B., Evstatieva, L., Oleszek, W., Aslani, M.R., Kostova, I. (2008). Distribution of steroidal saponins in Tribulus terrestris from different geographical regions. *Phytochemistry*. 69:176-86.

Dupont, S., Caffin, N., Bhandari, B. and Dykes, G.A. (2006). In vitro antibacterial activity of Australian native herb extracts against food-related bacteria. *Food Control*. 17: 929-32.

El-shazly, A., Abdel-All, M., Tie, A. and Wink, M. (1999). Pyrrolizidine alkaloids from *Echium rauwolfii* and *Echium horridum* (Boraginaceae). *Z. Naturforsch*, 54: 295-300.

Farnsworth, N.R., Akerele, O., Bingel, A.S., Soejarto, D.D. and Guo, Z. (1985). Medicinal plants in therapy. *Bulletin of the world health organization*. 63: 965-81.

Ghaffari, M.A., Bano, S. and Hayat, K. (2013). Antimicrobial and phytotoxic effects of the plant *Heliotropium dasycarpum* L. *Int. J. Pharm. Bio. Sci.* 4: 339-45.

Ioset, J.R., Marston, A., Gupta, M.P. and Hostettmann, K. (2000). Antifungal and *larvicidal cordiaquinones* from the roots of *Cordia curassavica*. *Phytochemistry*. 53: 613-17.

Ioset, J.R., Marston, A., Gupta, M.P. and Hostettmann, K. (1998). Antifungal and larvicidal meroterpenoid naphthoquinones and a naphthoxirene from the roots of Cordia linnael. *Phytochemistry*. 47: 729-34.

Joharchi, M.R. and Amiri, M.S. (2012). Taxonomic evaluation of misidentification of crude herbal drugs marketed in Iran. *Avicenna journal of phytomedicine*. 2: 105-112.

Khatamsaz, M. (1991). Tehran: Research Institute of Forests and Rangelands 50p.-illus. *Ara Icones, Keys. Geog.* 2: 50p.

Kumar, M., Agarwal, R.C., Dey, S., Rai, V.K. and Johnson, B. (2009). Antimicrobial activity of aqueous extract of Terminalia chebula Retz. On Gram-Positive and Gram-Negative micro-organisms. International Journal of current pharmaceutical research. 1: 56-60.

Mazhar, F., Khanum, R., Ajaib, M. and Jahangir, M. (2015). Potent AChE enzyme inhibition activity of Zizyphus oxyphylla: A new source of antioxidant compounds. *Pakistan journal of pharmaceutical sciences*. 28: 2053-59.

Modak, B., Rojas, M., Torres, R., Rodilla, J. and Luebert, F. (2007). Antioxidant activity of a new aromatic geranyl derivative of the resinous exudates from *Heliotropium glutinosum* Phil. *Molecules*. 12: 1057-63.

Mongelli, E.L., Desmarchelier, C.R., Coussio, J. and Ciccia, G.R. (1997). The potential effects of allelopathic mechanisms on plant species diversity and distribution determined by the wheat rootlet growth inhibition bioassay in South American plants. *Rev. Chil. Hist. Nat*. 70: 83-9.

Murray, C.K., Holmes, R.L, Ellis, M.W, Mende, K., Wolf, S.E., McDougal, L.K., Guymon, C.H. and Hospenthal, D.R. (2009). Twenty-five year epidemiology of invasive methicillin-resistant *Staphylococcus aureus* (MRSA) isolates recovered at a burn center. *Burns*. 35:1112-17.

Osungunna, M.O. and Adedeji, K.A. (2011). Phytochemical and antimicrobial screening of methanol extract of *Heliotropium indicum* leaf. *Journal of Microbiology and Antimicrobials*. 3: 213-16.

Phyta, Z.F., Li, T., Papageorgiou, V.P., Mellidis, A.S., Assimopoulou, N.A., Pitsinos, E.M. and Couladouros, E.A. (1998). Inhibition of topoisomerase I by naphthoquinone derivates. *Bioorg Med Chem*. 8: 3385-90.

Rahman, A.U., Choudhary, M.I. and Thomsen, W.J. (2001). Bioassay techniques for drug development. Harwood academic. *British library, UK*. : 14-25.

Ramakrishnan, G., Kothai, R., Jaykar, B. and Rathnakumar, T.V. (2011). *In vitro* Antibacterial activity of different extracts of leaves of *Colendia procumbens*. *International Journal of PharmTech Research*. 3: 1000-4.

Rios, J.L. and Recio, M.C. (2005). Medicinal plants and antimicrobial activity. Journal of ethnopharmacology. 100: 80-4.

Sánchez, D.S., Najera, G.L., Rivera, I.L., Dorado, O., Cisneros, M.G. and García, M.N. (2009). Antimicrobial activity of medicinal plants from the Huautla Sierra Biosphere Reserve in Morelos (México). *Polibotánica*. 28:213-25.

Scott, O.F. and Osho, A. (2012). Comparison of Antimicrobial Effects of *Mezoneuron benthamianum, Helitropium indicum* and *Flabellaria paniculata* on Candida species. *Journal of Microbiology Research*. 2:18-23.

Shah, S.M., Hussain, S., Khan, A.U., Shah, A.U., Khan, H., Ullah, F. and Barkatullah. (2015). Cytotoxic and phytotoxic actions of *Heliotropium strigosum*. *Toxicology and industrial health*. 31: 429-32.

Sharma, R.A., Singh, B. and Singh, D. (2009). Ethnomedicinal, pharmacological properties and chemistry of some medicinal plants of Boraginaceae in India. *Journal of Medicinal Plants Research*. 3: 1153-75.

Shinwari, Z.K., Gilani, S.S., Kohjoma, M., Nakaike, T. (2000). Status of medicinal plants in Pakistani Hindukush Himalayas. In Proceedings of Nepal–Japan Joint Symposium on Conservation and utilization of Himalayan Med. *Resour*. 6: 257-64.

Shukla, Y.N., Tandon, J.S., Bhakuni, D.S. and Dhar, M.M. (1969). Chemical constituents of the antibiotic fraction of *Arnebia nobilis*. *Cellular and Molecular Life Sciences*. 25: 357-8.

Singh, B. and Singh, S. (2003). Antimicrobial activity of terpenoids from *Trichodesma amplexicaule* Roth. *Phytotherapy Research*. 17: 814-6.

Sofowora, A. (1994). Medicinal Plants and Traditional Medicines in Africa. *Chichester John, Wiley and Sons New York*. pp256.

Srivastava, S., Khare, A. and Khare, M P. (1991). A cardenolide tetraglycoside from *Oxystelma esculentum. Phytochemistry*. 30(1): 301-303.

Tandon, H.D., Tandon, B.N. and Mattocks, A.R. (1978). An Epidemic of Veno-occlusive Disease of the Liver in Afghanistan. *American Journal of Gastroenterology*. 70: 607-13.

Tareen, R.B., Bibi, T., Khan, M.A., Ahmad, M., Zafar, M. and Hina, S. (2010). Indigenous knowledge of folk medicine by the women of Kalat and Khuzdar regions of Balochistan, Pakistan. *Pak J Bot.* 42:1465-85.

Torres, R., Villarroel, L., Urzua, A., Delle-Monache, F., Delle-Monache, G. and Gacs-Baitz E. (1994). Filifolinol, a rearranged geranyl aromatic derivative from the resinous exudate of Heliotropium filifolium. Phytochemistry. 36: 249-50.

Trease, G.E. and Evans, W.C. (1989). A Textbook of Pharmacognosy. Baillere Tinall Ltd, *London*. 53.

Tschesche, R. (1971). Advances in the chemistry of antibiotic substances from higher plants. Springer, Berlin, Heidelberg. *Pharmacognosy and Phytochemistry*. 274-289.

Ullah, F., Malik, S. and Ahmed, J. (2009). Antibiotic susceptibility pattern and ESBL prevalence in nosocomial Escherichia coli from urinary tract infections in Pakistan. *African Journal of Biotechnology*. 8: 3921-26.

Urzúa, A., Echeverría, J., Rezende, M.C. and Wilkens, M. (2008). Antibacterial Properties of 3 H-Spiro [1-benzofuran-2, 1'-cyclohexane] Derivatives from *Heliotropium filifolium*. *Molecules*. 13: 2385-93.