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ORIGINAL ARTICLE

Screening of Foeniculum Vulgare L, Coriandrum Sativum L, Pegnum Harmala L. and Achilleae Millefolium L Collected from Balochistan Against Fungi Causing Onychomycosis

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ABSTRACT

Onychomycosis is referred to as fungal nail infection or toe nail infection generally caused by Trichophyton, Candida albicans , Microsporum and Aspergillus species. The Aim of the study was to investigate antifungal potential of four medicinal flora of Balochistan namely, Foeniculum vulgare L. , Coriandrum sativum L. **,** Pegnum harmala L. and Achilleae milefolium L. against causative fungal strain of nail infection. Different fractions were prepared using different solvents such as hexane, chloroform and ethyl acetate and antifungal activity was determined through agar well diffusion method against fungal strain candida albicans. For the qualitative determination of phytochemical composition different tests were carried out including tests for alkaloids, terpenoids , carbohydrates, glycosides, proteins, steroids, saponins, phenols and tannins. The highest inhibition zone were observed in aqueous 80% crude methanolic extracts of Foeniculum vulgare L. i.e 26 mm and Coriandrum sativum L. 30mm respectively. Aqeous layer of the crude extract of both plants showed potent activity that were 24 mm and 25mm for Foeniculum L. vulgare and Coriandrum sativum L.. the ethylacetet fractions showed inhibition zone 19mm and 18mm in Foeniculum vulgare L. and Coriandrum sativum L. Chloroform and hexane fractions showed least activity against fungi causing onychomycosis. An Antifungal activity highest inhibition zones were observed in aqueous 80% crude methanolic extracts of Pegnum harmala L. i.e 39 mm and Achilleae millefolium 34 mm respectively. Aqeous layer of the crude extract of both plants showed potent activity that were 32 mm for Pegnum harmala L. and 25mm for Achilleae millefolium L. The choloroform fractions showed inhibition zone 19mm in Pegnum harmala L. while 16mm in Achilleae millefolium L. Hexane and ethyl acetate fractions of Pegnum harmala L. and Achilleae millefolium L. showed least activity against fungi causing onychomycosis.

Keywords: Onychomycosis , Phytochemical composition , Candida albicans, Agar well diffusion, Antifungal activity.

INTRODUCTION

The use of medicinal plants to cure diseases and release sufferings is a common practice that was started from the earliest times of mankind's history till the use of plants as a source of medicine is very much important for human beings (Hill, 1989 , Kultur, 2007). A human fungal infection poses serious medical issues. Up to now, more than a hundred thousand fungal species are considered as natural contaminants (Kacaniova, 2003). There is a general consensus among researchers, clinicians and pharmaceutical companies that new, potent, effective and safe antifungal drugs are needed (Selitrennikoff, 1992). Therefore the medicinal plants play an important role for the treatment of both human and animal fungal diseases and are important source for the discovery of new antifungal drugs (Mathias-Mundy and McCorkle 1995).

The term Onychomycosis is referred to as fungal infection of fingernails or toenails. It is not deadly but it can cause pain, discomfort and deformity. Different forms of skin infections are stated to as dermatomycoses generally caused by Fungi, viruses, bacteria and yeasts. The most common harmful species of fungi are Candida albicans, Epidermophyton, Microsporum and Trichophyton spp. Mucocutaneous fungal disease in humans caused by Candida albicans which is a major component of the normal skin flora (Hay, 1993).

It was reported that 20% of person have onychomycosis in between 40 to 60 age. (veer et al, 2007: Loo, D. S. 2007) 12.7 % have onychomycosis due to candida albicans out of 102 renal patients (Güleç et al, 2003).

Factors causing onychomycosis are HIV infection, use of antibiotics, chronic smoking, and exposure to fungus and trauma to aged nails (Lange 2006). It was recorded that out of five patients one have fungus disease having Hiv disease (Gupta et al 2000). Laborers , farmers and athlets often get onychomycosis. (veer et al, 2007)

Mostly three kinds of fungi are associated in onychomycosis that are, candida albicans, nondermatophytic molds and dermatophytes. Depends on host immunity, when immunity is weak candida can stimulate infection from less superficial lesion to dangerous illness. Recently it is reported that onychomycosis by candida increased and it is found that all forms of onychomycosis are caused by candida. (veer et al, 2007)

Foeniculum vulgare is medicinal plants having importance in cosmetics, food and pharmeaceutical and health care industries (Abe & Ohtani, 2013). Foeniculum vulgare is seasonal herb. Origin of This plant is from southern Mediterranean region and through cultivation it is grown throughout eastern, northern and western hemisphere, especially in Asia. It is grown wild and also cultivated in field. For its edible seeds romans grew it (Krishnamurthy, 2011). Foeniculum vulgare is medicinal plant belongs to family Apiaceae (muckenstrum et al., 1997). Fennel is known by more than 100 names. Studies showed that Foeniculum vulgare efficiently control infections of fungi, bacteria and virus (kour & arora, 2009; Orhan et al., 2012; Morales et al., 2012). Use of Foeniculum vulgare is not harmful, even if it is used daily in any form such as boiled, baked, grilled and raw form.

Coriandrum is important herb, used as spice and used for flavoring. This genus Coriandrum has two species. It is available throughout the year. Originated from Mediterranean cultivated especially in tropical areas. It is a tropical crop needs cooled, free climate and dry habitat for growth especially for germination time. Optimal temperature for growth is 20 to 25 ˚c (Varghese, 2001). Seeds are used as antimicrobial agents, and used to cure nausea, seasonal fever, and cold and stomach infection, in household medicines. Coriandrum sativum is known for its antioxidant activity (Nazni & Dharmaligam, 2014).

Pegnum harmala L. which is commonly known as Syrian rue and Wild rue is a flowering plant and is commonly distributed in the Central Asia, North Africa, Middle East, America, and Australia and in part of South mainly in India and Pakistan. This plant is known as “Harmal” in North Africa and (African Rue) in United States (Mahmoudian et al., 2002). It belongs to family Zygophyllaceae in the order of Zygophyllales that contains about 22 genera and more than 250 species.

The yarrow, Achilleae millefoilum L. is an important specie of Asteraceae family with common utilization in folk medicine of many countries. It is perennial, erect, aromatical, and herbaceous plant of 30-50cm of height. It occurs of native form in the Europe, North America, South of Australia and North of Asia (Mil-folhas, 2003) and widely cultivated in almost all Brazil for being perfectly suitable to the climate (Lorenzi et al., 2002; Martins et al., 2000; Panizza, 1997).

Candida albicans: Candida albicans can also infect fingernails producing onychomycosis and paronychia and is more common with advanced human immunodeficiency virus (HIV) disease (Conant, 1994).

MATERIALS AND METHODS

Sample collections: The study of plants were carried out in the region of Balochistan Pakistan. The four plant samples that were used in this study named as seeds of Foeniculum vulgare L., Coriandrum sativum L., Pegnum harmala L. whole plant of Achilleae millefolium L. The plants were collected from Balochistan that were Balochistan Agricultural Research and Development Center Quetta (Arid zone).

The whole plant of Achilleae millefolium L. was shade dried at room temperature for ten days. The dried materials Foeniculum vulgare L., Coriandrum sativum L., Pegnum harmala L. and whole plant of Achilleae millefolium L. were crushed to get powdered mass. The powdered materials were soacked in 80% methanol and kept it for fifteen days with manual shaking normally from time to time. The supernatant from suspended materials were concentrated using rotary evaporator to obtain gummy crude extract of Foeniculum vulgare L., Coriandrum sativum L., Pegnum harmala L. Achilleae millefolium L. The crude extract of seeds of Foeniculum vulgare L. (F-80M), Coriandrum sativum L. (C-80M), Pegnum harmala L. (P-80M) and Achilleae millefolium L. (A- 80M) were suspended in distilled water. The homogenous suspension was then subjected to liquid - liquid extraction with different organic solvents such as hexane, choloroform and ethyl acetate to get the corresponding fractions (F-hex, F-ch, F-eth and F-aq) (C-hex, C-ch, C-eth and C-aq) (P-hex , P-ch , P- eth and P- aq) (A- hex , A-ch , A- eth and A- aq) respectively as shown in (Figure 3.1 and 3.2).

Qualitative Phytochemical Analysis: The extract was tested for the presence of bioactive compounds by using following standard methods (Abayomi, S.,1982 ; Trease, G.E., Evans, W.C. 1989 ; Harborne, J.B. 1984).

Test for Alkaloids: 2ml of 1% hydrochloric acid (HCl) mixed with crude extracts then heated gently. In mixture the Mayers and Wagners reagents were added.

Test for Terpenoids: 2 ml of chloroform dissolved in the plant crude extract kept for evaporation until its dryness then added the 2 ml of concentrated sulphuric acid (H_2SO_4) and heated about for two minutes.

Test for Steroids: Firstly added the 2 ml of chloroform in the crude extracts and carefully add the concentrated Sulphuric acid (H_2SO_4) to side walls of test tubes.

Carbohydrates (Benedict’s Test): 2ml of Benedicts reagents mixed with plant crude extract and then heated .

Glycosides (Salkowski’s Test): First of all added the 2ml of chloroform in the plant crude extract. Then add carefully 2ml of concentrated sulphuric acid (H_2SO_4) and shaken smoothly or lightly.

Proteins (Ninhydrin Test): The plant crude extract mixed with 2ml of 0.2% solution of Ninhydrin when boiled.

Test for Phenols and Tannins: 2ml of 2% solution of ferric chloride $FeCl_3$ was mixed with plant crude extract.

Test for Saponins: Firstly plant extract was added with 5ml of distilled water in a test tube and shake it vigorously. You may add the olive oil and then shake it for better result.

Agar well diffusion assay: Agar well diffusion method was used for antifungal susceptibility test described by National Committee for Clinical Laboratory Standards (2003). The agar well diffusion method was used for antifungal assay. The fungal strain Candida albicans was used for antifungal activity causing onychomycosis. The Sabouraud Dextrose agar (SDA) was prepared for yeast at 4 ˚C. Candida albicans was cultured on Sabouraud

Dextrose Agar for isolation and incubated at 37 ˚C for 24hrs.

For the preparation of the Inocula colonies of fungi were mixed with physiological saline and the turbidity were corrected by adding sterile saline until a Mc farland turbidity standard 0.5. Petri plates (150mm ×15mm) were prepared by pouring 60ml SDA for Candida albicans and allowed to solidify. Petri plates were dried and 1ml of standardized inoculum was poured and uniformly spread. The excesss Inocula was drained and allowed to dry for fifteen minutes.

Several Equidistant wells were made in the medium using a sterile cork borer (6mm in diameter) and 50µl of the methanolic extracts (100 mg/ml) diluted in DMSO 2% were placed into the wells and incubated for 24 hrs at 37˚C for C. albicans. Amphotericin B (0.2mg/ml) was dissolved in DMSO and served as positive control. The tests were carried in triplicate. The antifungal activity was measured as the diameter (mm) of clear zone of growth inhibition.

Natural products play an important role in the drug discovery and development of plants. The fungi are recognized as a source of natural products. The medicinal plants having the greater role to control such kind of diseases such as skin infection dermatophytes and fungi causing disease on nails like onychomycosis.

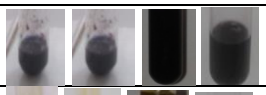
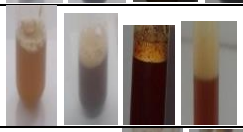
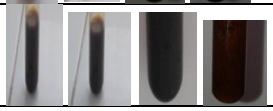
The present research demonstrated that the 80% crude methanolic extracts of four plant species from Balochistan that were studied for their qualitative phytochemical analysis and for antifungal potential. The four active extracts of the plant Foeniculum vulgare L., Coriandrum sativum L. ,Pegnum harmala L. and Achilleae millefolium L., demonstrated the presence and absence of common phytoconstituents like alkaloids , flavonoids, glycosides, steroids, saponins, phenols and tannins. The results were shown below in (Table 4.1 and 4.2).

RESULTS AND DISCUSSION

In the present research the crude 80% aqueous methanolic extract of four plants from Balochistan were studied for their antifungal activity. The studies revealed that the selected plants extracts showed excellent antifungal potential against Candia albicans. Various fractions of these crudes extracts of each plant were prepared using various s**olvent**s. The antifungal activity of Foeniculum vulgare, Coriandrum sativum, Pegnum harmala and Achilleae millefolium was tested using agar well diffusion method by measuring inhibition zone **in mm.**

4.1 Qualitative Phytochemical Analysis of Plants: The Foeniculum vulgare, Coriandrum sativum Pegnum harmala and Achilleae millefolium were analyzed for the presence or absence of various phytochemicals present in alkaloids, proteins, phenols and tannins, glycosides, terpenoids steroids, saponins, Ninhydrin and terpenoids Confirmed by the qualitative tests. Foeniculum vulgare showed positive results for all test mentioned above except terpenoids whereas Coriandrum sativum showed negative result for Salkowski's test. Pegnum harmala showed positive results for all test mentioned above except carbohydrates, proteins and phenols and tannins. Whereas Achilleae millefolium showed negative result for alkaloids test.

Table 4.1: Qualitative Phytochemical screening of the seeds of Foeniculum vulgare L., Coriandrum sativum L. and Punica granatum

Compound name	Foeniculum vulgare	Coriandrum sativum	Pegnum harmala	Achilleae millefolium	Representative color
Ninhydrin (protein test)	+ve	+ve	-ve	+ve	
Benedict (carbohydrates)	+ve	+ve	-ve	+ve	
Salkowski's (glycosides)	+ve	-ve	+ve	+ve	
Phenol and tannin	+ve	+ve	-ve	+ve	
Saponins	+ve	+ve	+ve	+ve	
Terpenoids	-ve	+ve	+ve	+ve	
Steroids	+ve	+ve	+ve	+ve	
Alkaloid	+ve	+ve	+ve	-ve	

4.2 Antifungal Activity of the Plant Extracts: Antifungal activity of plant Foeniculum vulgare, Coriandrum sativum, Pegnum harmala and Achilleae millefolium were analyzed against a fungus strain Candida albicans causing onychomycosis. All the samples showed antifungal activity but there was difference in inhibition zones due to the variation of phytochemical composition. Highest antifungal activity was found in methanolic crude extract (F-80M) and aqueous extract (F-Aq) of Foeniculum vulgare with inhibition zone of 26mm and 24mm respectively, whereas its ethyl acetate (F-Et) and hexane (F-Hex) fractions showed moderate activity with inhibition zone of 19mm and 17mm respectively. Chloroform (F-Ch) fractions showed least inhibition zone against fungi causing onychomycosis that is 13mm**.** The crude methanolic extract of Coriandrum sativum (C-80M) and its aqueous fractions (C-Aq) showed highest activity with inhibition zone of 30mm and 25mm respectively and moderate activity was shown by ethyl acetate (C-Et) fraction and chloroform (C-Ch) fraction that is 18mm and 16mm respectively. Least inhibition zone was shown by hexane fraction (C-Hex) with inhibition zone of 12mm. Highest antifungal activity was found in methanolic crude extract (P-80M)and aqueous extract (P-Aq) which was 39mm and 32mm. Moderate activity was shown by chloroform fractions (P-Ch) and ethyl acetate fraction (P-Eth) which was 19mm and 16mm. Least inhibition zone was shown by hexane fraction (P-Hex) which was 12mm. Highest antifungal activity was found in methanolic crude extract (A-80M)and aqueous extract (A-Aq) which was 34mm and 25mm. Moderate activity was shown by chloroform fractions (A-Ch) and hexane fraction (A-Hex) which was 16mm and 15mm. Least inhibition zone was shown by ethyl acetate fraction (A-Eth) which was 12mm.

Table 2: Inhibition zones of Foeniculum vulgare L. , Coriandrum sativum L. , Pegnum harmala L. , and Achilleae millefolium L.

S.No	Fraction	Zone of inhibition of Pegnum harmala L. in mm	Zone of inhibition of Achilleae millefolium in mm	Zone of inhibition of Foeniculum vulgare in mm	Zone of inhibition of Coriandrum sativum in mm
1	DMSO	0mm	0mm	0mm	0mm
2	crude (80%)	39mm	34mm	26mm	30mm
3	Hexane	12mm	15mm	17mm	12mm
4	Chloroform	19mm	16mm	13mm	16mm
5	Ethyl acetate	15mm	12mm	19mm	18mm
6	Aqueous extract	32mm	25mm	24mm	25mm

DISCUSSION

In my study I investigated antifungal activity and phytochemical screening of four species Foeniculum vulgare, Coriandrum sativum, Pegnum harmala and achiella millefolium. Antifungal activity was evaluated against fungi causing Onychomycosis. The seeds of Foeniculum vulgare, Coriandrum sativum, Pegnum harmala and Achilleae millefolium was collected from areas of arid zone (Balochistan agriculture research and development center Quetta) and ziarat of province Balochistan.

According to Jamwal et al., (2013) evaluated the phytochemical analysis of Foeniculum vulgare methanolic extract. In his study test for flavonoids and saponins showed +ve results and alkaloids and terpenoids showed –ve results. While in my study ninhydrin test, benedict test, salkovisky, phenol, saponins, steroids, alkaloids showed +ve results. Only terpenoid showed –ve result.

According to Rahimi et al., (2013) Foeniculum vulgare showed antifungal activity against Aspergillus, Candida albicans and dermatophyte.

According to Kotti et al., (2015) ethanolic and aqueous extracts of Foeniculum vulgare seeds antioxidant activity was calculated by free radical scavenging, total antioxidant, metal chelating activity and hydrogen peroxide scavenging method.

According to Shahat et al., (2011) aqueous and ethanolic extract of fennel seeds displayed 77.5% and 99.1% antioxidant activity. While in my study 80% methanolic seed extract showed inhibition zone of 26mm against Candida albicans isolated from onychomycosis patient.

According to Madhavan and Tharakan (2017) for determination of plants biological component phytochemical examination is useful. Qualitative analysis of Coriandrum sativum seeds displayed the presence of alkaloids, phenolic compounds, saponins and flavonoids. Phytochemicals possess anti-inflammatory activity like terpenoids, saponins, steroids, tannins, alkaloids and flavonoids.

According to Rajeshwari et al., (2012) phytochemical in methanolic extract of Coriandrum sativum, phenolic compounds, tannins, saponins, flavonoids, alkaloids and glycosides showed +ve results and terpenoids and steroids showed –ve results. While in my research phenol, saponins, terpenoids, steroids, alkaloids, benedict test and ninhydrin test showed +ve results and salkovisky showed –ve results.

According to Saeed and Perween (2007) decoction of Coriandrum sativum did not displayed antifungal activity against Candida albicans. According to Al-Ebady and Ali., (2010) Coriandrum sativum, Eugenia caryophyllus and Cyperus rotundus ethanolic extract exhibited various antifungal activity against Candida albicans. Coriandrum sativum showed no effect against Candida albicans. Study indicated that there is relation between concentration of plant used and zone of inhibition against fungus strains grown in Sabourauds dextrose agar, when plant sample concentration increase it will increase zone of inhibition. While in my study ethyl acetate fraction showed antifungal activity against Candida albicans isolated from onychomycosis patient.

Pegnum harmala seeds have been considered from ancient time to date as a plant with drug usages regarding to some alkaloids compounds such as harmalin and

harmalol. The compounds extracted from this plant have shown different medical characteristics such as anti inflammation effects that were reported by (El-Saad El Rifaei ,.1980).

Among the five medicinal plants reported by Hashem (2011) against dermatophytes which included Artemisia judaica, Ballota undulate, Cleome amblyocarpa, Pegnum harmala and Teucrium polium the ethanol extract of Ballota undulate was the most effective. With studies of an antifungal activity of Pegnum harmala L. shows the highest activity against fungi causing onychomycosis.

Studies of (Mahmoudian et al., (2002) shows that the Peanum harmala contains several alkaloids which are found especially in the seeds and the roots. These alkaloids include B carbolines such as harmine, harmaline (identical with harmidine) harmalol and harman and quinazoline derivates vasicine and vasicinone.

The whole plant of Achilleae millefolium L. was selected for the isolation of fungi on the basis of medicinal importance and availbility. The fungi isolated from the Achilleae millefolium L . were identified as Candida albicans. In Achilleae millefolium L inhibition zones were observed in aqueous 80% crude methanolic extracts that were 34 mm respectively.

CONCLUSION

The current research was conducted to study the presence of different phytochemical as well as antifungal potential of Foeniculum vulgare, Coriandrum sativum Pegnum harmala and Achilleae millefolium against infective disease caused by Candida albicans. Both plants and their seeds were collected from Quetta zone. This research revealed that the seeds of these two plants have potent inhibitory effects against Candida albicans. Among various extracts and fractions the finest outcome was showed by crude extract (80%) of these plants and aqueous extract of plants.

Recommendation: The detail phytochemical investigation can be carried out to isolate the active constituents and extraction and fractionation can be done using different parts of the plants. Further phytochemical investigations can be carried out on the essential oil extracts of Foeniculum vulgare, Coriandrum sativum and Punica granatum to isolate and identify the active compound responsible for their antimicrobial, antioxidant and low cytotoxic properties to get a lead antibiotic.

REFERENCES

1. Abe R, Ohtani K.. An ethnobotanical study of medicinal plants and traditional therapies on Batan Island, the Philippines. Journal of Ethnopharmacology. 2013; 145(2), 554-565.
2. El-Rifaie MES. Peganum harmala: its use in certain dermatoses. International Journal of Dermatology. 1980; 19(4), 221-222.
3. Güleç A., Demirbilek M, Seçkin D. Can F, Saray Y, Sarifakioğlu E, Haberal M. Superficial fungal infections in 102 renal transplant recipients: a case-control study. Journal of the American Academy of Dermatology. 2003; 49(2), 187-192.
4. Gupta AK, Taborda P, Taborda V, Gilmour J, Rachlis A, Salit I, Summerbell RC. Epidemiology and prevalence of onychomycosis in HIV-positive individuals. International journal of dermatology. 2000; 39(10), 746-753.
5. Harborne JB. 1984. Methods of plant analysis. In Phytochemical methods. Springer, Dordrecht. 2008; (pp. 1-36).
6. Hashem M. Antifungal properties of crude extracts of five Egyptian medicinal plants against dermatophytes and emerging fungi. Mycopathologia.2001; 172(1), 37-46.
7. Hay RJ. Onychomycosis: agents of choice. Dermatologic clinics. 1993;11(1), 161-169.
8. Hill AF. Economic botany. A textbook of useful plants and plant products. Economic botany. A textbook of useful plants and plant products. 1953; (2nd edn).
9. Jamwal NS, Kumar S, Rana AC. Phytochemical and pharmacological review on Foeniculum vulgare. An International Journal of Pharmaceutical Sciences. 2013; 4(3). 327-341.
10. Jayatilake JAMS, Tilakaratne WM, Panagoda GJ. Candidal onychomycosis: a mini-review. Mycopathologia. 2009;168(4), 165-173.
11. Kacaniova, M. (2003). Feeding soybean colonization by microscopic fungi. Trakya Univ. J. Sci, 4(2), 165-168.
12. Kaur GJ, Arora DS. Antibacterial and phytochemical screening of Anethum graveolens, Foeniculum vulgare and Trachyspermum ammi. The Official Journal of the International Society for Complementary Medicine Research. 2009; 9(1), 30.
13. Kooti W, Ghasemiboroon M, Asadi-Samani, M, Ahangarpoor A, Zamani M, Amirzargar A, Hardani A. The effect of halcoholic extract of celery leaves on the delivery rate (fertilization and stillbirths), the number, weight and sex ratio of rat off spring. Advances in Environmental Biology. 2014;8(10), 824-831.
14. Krishnamurthy KH. Medicinal plants: Madhurika, saunf or fennel (Foeniculum vulgare, Gaertn). Journal of New Approaches to Medicine and Health. 2001; 19(1), 1-4.
15. Lange M, Roszkiewicz J, Szczerkowska-Dobosz A, Jasiel-Walikowska E, Bykowska B. Onychomycosis is no longer a rare finding in children. Mycoses. 2006; 49(1), 55-59.
16. Loo DS. Onychomycosis in the elderly. Drugs & aging. 2007; 24(4), 293-302.
17. Lorenzi H, Matos FJ. Plantas medicinais no Brasil: nativas e exóticas.2002.
18. Madhavan M, Tharakan ST. Study on phytochemicals, total phenols, antioxidant, anthelmintic activity of hot water extracts of Coriandrum sativum seeds. World Journal of Pharmacy and Pharmaceutical Science. 2017; 6(8), 2519-2527.
19. Mahmoudian M, Salehian P, Jalilpour H. Toxicity of Peganum harmala: review and a case report.2002.
20. Mathias-Mundy E, McCorkle CM. Ethnoveterinary medicine and development-a review of the literature. 1995;12-19.
21. Morales P, Carvalho AM, Sánchez-Mata MC, Cámara M, Molina M, Ferreira IC. Tocopherol composition and antioxidant activity of Spanish wild vegetables. Genetic Resources and Crop Evolution. 2012; 59(5), 851-863.
22. Muckensturm B, Foechterlen D, Reduron JP, Danton P, Hildenbrand M. Phytochemical and chemotaxonomic studies of Foeniculum vulgare. Biochemical Systematics and Ecology.1997; 25(4), 353-358.
23. Nazni P, Dharmaligam R. Isolation and separation of phenolic compound from coriander flowers. International Journal of Agricultural and Food Sciences. 2014; 4(1), 13-21.
24. Orhan İE, ÖZÇELİK B, Kartal M, Kan Y.Antimicrobial and antiviral effects of essential oils from selected Umbelliferae and Labiatae plants and individual essential oil components. Turkish Journal of Biology. 2012; 36(3), 239-246.
25. Rahimi R, Ardekani MRS. Medicinal properties of Foeniculum vulgare Mill. in traditional Iranian medicine and modern phytotherapy. Chinese Journal of Integrative Medicine. 2013; 19(1), 73-79.
26. Rajeshwari CU, Shobha RI, Andallu B. Phytochemicals and medicare potential of ethyl acetate fraction of methanolic extract of coriander (Coriandrum sativum L.) seeds. Annals of Phytomedicinal an Intrnational Journal. 2013; 2(2), 63-70.
27. Saeed S, Tariq P. Antimicrobial activities of Emblica officinalis and Coriandrum sativum against gram positive bacteria and Candida albicans. Pakistan Journal of Botany, 2007; 39(3), 913-917.
28. Selitrennikoff, C. P. (1992). Screening for antifungal drugs. Biotechnology (Reading, Mass.), 21, 189-217.
29. Shah FA. Phytochemical Screening of Achilliea Millefolium Collected at IIIM Srinagar (J&K) 2018.
30. Shahat AA, Ibrahim AY, Hendawy SF, Omer EA, Hammouda FM, Abdel-Rahman FH, Saleh MA. Chemical composition, antimicrobial and antioxidant activities of from organically cultivated fennel cultivars. Molecules. 2011; 16(2), 1366-1377.
31. Souza TM, Rangel VLBI, Pietro RCLR, Santos LE, Moreira RRD. Phytochemical screening of Achillea millefolium harvested at Araraquara–SP. Rev Bras Plant Med Botucatu. 2006; 8, 151-154.
32. Trease GE, Evans WC. Pharmacognosy. 13th. ELBS/Bailliere Tindall, London. 1989; 345-6.
33. Veer P, Patwardhan NS, Damle AS. Brief Communications-Study of onychomycosis: Prevailing fungi and pattern of infection. Indian Journal of Medical Microbiology. 2007; 25(1), 53-56.
34. Verghese J. Coriander. Indian Spices.2001; 38(8).