RESEARCH ARTICLE Am. J. PharmTech Res. 2014; 4(5) ISSN: 2249-3387

AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Quercetin Level in Stem and Flower Extracts of Clitoria Ternatea and its Antidermatophytic Potential

Rajalalakshmi PadmaVairavasundaram*[1], Kalaiselvi senthil[1]

1. Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam institute for Home science & Higher education for Women, Coimbatore. Tamil Nadu, India

ABSTRACT

Clitora ternatea is a disease resistant plant used in ethno-pharmacological preparations for its high therapeutic value of primary and secondary metabolites. The aim of this study was to investigate the amount of quercetin in plant parts of *Clitoria ternatea* and its antifungal activity on dermatophytes. To obtain chemical pattern of flavonoids and quercetin in different plant parts, the extraction solvent was standardized and a finger printing profile of flavonoid was established by means of high performance liquid chromatography (HPLC). The greatest amount of flavonoid was observed in methanolic extracts of *C.ternatea* flowers while the lowest level was found in the *C.ternatea* stem extracts, whereas HPLC fingerprinting of flavonoids in flowers was significantly different from stem and leaf extracts. These extracts also differ in their fungicidal capacity against dermatophytes tested, although they all showed significant antifungal activity against *Candida sp.*. and *Aspergillus sp.*. flower extracts showed concentration dependent strong inhibition on growth of *Microsporum gypseum* and *Epidermophyton floccosum*, and weak inhibition on *Trichophyton metagraphytes*. The results of this study will be used to promote application of *C.ternatea* for improving human health by providing antidermatophytic effect on skin infections.

Keywords: Quercetin, *Clitoria ternatea*, HPLC, Antidermatophytic, Flavonoids.

*Corresponding Author Email: pvrajivicas@gmail.com
Received 18 September 2014, Accepted 22 September 2014

Please cite this article in press as: Rajalalakshmi PV *et al.*, Quercetin Level in Stem and Flower Extracts of Clitoria Ternatea and its Antidermatophytic Potential . American Journal of PharmTech Research 2014.

INTRODUCTION

Clitoria ternatea is an ornamental drought tolerant plant mainly occurs in India, Africa and United states, which is traditionally used as medhya rasayana in Ayurveda. Leaves of *C.ternatea* have been reported to have analgesic, anti-inflammatory[1], hypoglycemic[2], antioxidant and hepatoprotective activity[3]. Secondary metabolites such as phenolic compounds play an important role in plant defense mechanisms, which is accumulated on aerial parts of the plant during microbial infections and most of them are known to be valuable in human health[4]. Several phytopharmaceutical compounds have been identified and quantified in *C.ternatea* samples, these included taraxerol[5], flavonol glycosides[6] and delphidin[7]. Flower extracts have proven to the potent antioxidant and protected erythrocytes against hemolysis[8] and liver damage[9]. Apart from protective effects of flavonoids, this class of plant metabolites is upcoming area of anti-infective research, which has potent antiviral and antifungal activity. Fungal infections are common in hot and humid climatic countries like India; dermatophytes are a group of filamental fungi exclusively infect keratin rich host structures such as nails and skin[10]. After germination on infected skin, these fungi produce proteolytic enzymes and invade keratinized structures by digesting keratin in to short peptides and amino acids[11]. Photodynamic treatment using UV light[12] and topical application of antifungal agents such as clotrindazole, and miconazole[13] are used to treat dermatophytosis for over ten years. But side effects of antibiotics in humans and microbial resistance towards antibiotics stimulated requirement of new antifungal agents from plant sources. Plants endow with crucial compounds with active principles, having least side effects, and proven to be useful for microbial control[14]. Seeds of *clitoria ternatea* are well-known for antifungal activity; an antifungal protein isolated from seeds is homologous to plant defensins[15]. Previous reports on *C.ternatea* leaves showed antifungal effect only towards *Aspergillus niger*[16] and there are no reports available on its antidermatophytic potential. So the present investigation was undertaken to examine quercetin level in extracts and to evaluate its antifungal activity towards dermatophytes.

MATERIALS AND METHODS

Plant material

Fresh leaves, stems and flowers of *C.ternatea* were collected from Tiruchengode area, TamilNadu, India and authenticated at Botanical survey of India, Coimbatore, TamilNadu. Each plant part was shade dried and ground in to coarse powder and active principle was extracted using maceration method with solvents of different polarity (Petroleum ether, Benzene, Chloroform, Acetone, Ethanol and Methanol). After extraction, extracts were centrifuged; supernatants were collected, dried and estimated for extractive value.

Estimation of Flavonoids

One gram of each extract powder was re-dissolved in methanol and the amount of flavonoid in extracts estimated using standardized colorimetric method[17]. The total flavonoid content was expressed in terms of Quercetin Equivalents (QE).

Estimation of Quercetin by HPLC

Extracts with high flavonoid content was subjected to HPLC analysis, first detection and estimation of standard quercetin was optimized at concentration from 0.1- 10mg/ml. Then quercetin from extracts was separated by using C18 column and mobile phase standardized as methanol and 0.5% phosphoric acid (60:40) and peaks were observed at 254nm at flow rate 1ml/min. Crude extract powders of *C.ternatea* (0.1g) was dissolved in HPLC grade methanol and used for analysis.

Antifungal activity

Microorganisms

Eleven different fungal strains including dermatophytes were isolated from patients with various skin infections and identified by Department of Microbiology, Vivekanandha college of Arts and Sciences for women, Tiruchengode. Each fungal species were maintained on Sabouraud's agar and spore germination was observed and spores were diluted to 1 X 10^7CFU/ml and used for further antifungal studies.

Disc diffusion method

Selected fungal strains (1 X 10^5CFU/ml) were inoculated separately on Sabouraud's agar plates and used for disc diffusion method[18]. Crude *C.ternatea* extracts at different concentrations (50, 100, 200µg/ml respectively) were dissolved in Dimethyl sulfoxide and Sterile filter paper discs were impregnated with these extracts were kept on the culture plate and incubated at 27^0C. Zone of inhibition (mm) was measured after 48hrs. Quercetin and Ketaconazole were used for these experiments as positive controls.

Minimum Fungicidal concentration (MFC)

The effects of *C.ternatea* crude extracts in relation to concentration gradient on fungal strains were determined by broth dilution method[18]. Each Fungal suspension (1 X 10^7CFU/ml) was inoculated into separate Sabouraud's broth, which were incubated in presence of various concentrations (0.05-2.0mg/ml) of plant extracts. Growth of fungal strains was observed after 48hrs and minimum concentration required for strong inhibition on growth was noted. Six replicates were used for each concentration and experiments were repeated thrice.

RESULTS AND DISCUSSION

Extraction of active metabolites

Plant parts of *C.ternatea* were extracted for antidermatophytic substances using six different solvents by employing maceration method. The extraction yield (i.e extractive value) of solvents on each plant part is given in Figure.1. Of the solvents used for extraction, methanol yielded the highest amount of extraction (5.72g/100g) and benzene exhibited the lowest level of extraction (0.78g/100g). The extraction efficiency of the solvents increased in the order benzene < petroleum ether < chloroform< acetone< ethanol<methanol.

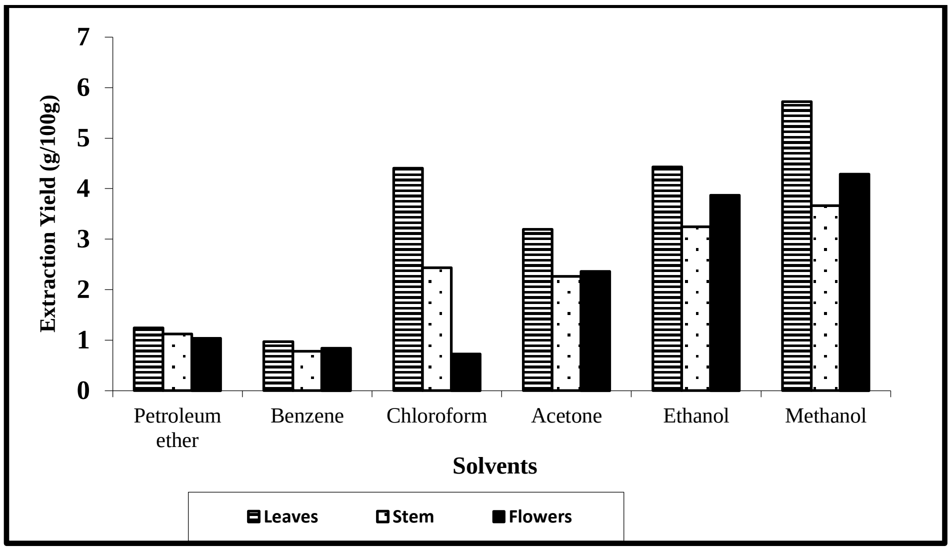

Figure.1. Effect of solvents on extraction yield of *C.ternatea* plant parts

Total flavonoids content

Flavonoids present in plant parts such as flowers, fruits and seeds, which have numerous health benefits particularly with respect to their reported antimicrobial properties[19]. Such flavonoids can be extracted from *C.ternatea* plant parts using variety of solvents with different polarity, and it is shown in Figure.2. Among six solvents used, methanol extracts exhibited significantly highest flavonoid content (43.56mg/g) as compared to other solvents used. Extraction efficiency of chloroform and benzene was lower and there was no significant result obtained in their flavonoid content. Irrespective of the effect of solvents, it was found that flower extracts of *C.ternatea* contain most of the flavonoids (43.56mg/g) followed by leaf (37.12mg/g) and stem extracts (24.67mg/g). Methanolic extracts which showed greatest flavonoid content used for further HPLC analysis.

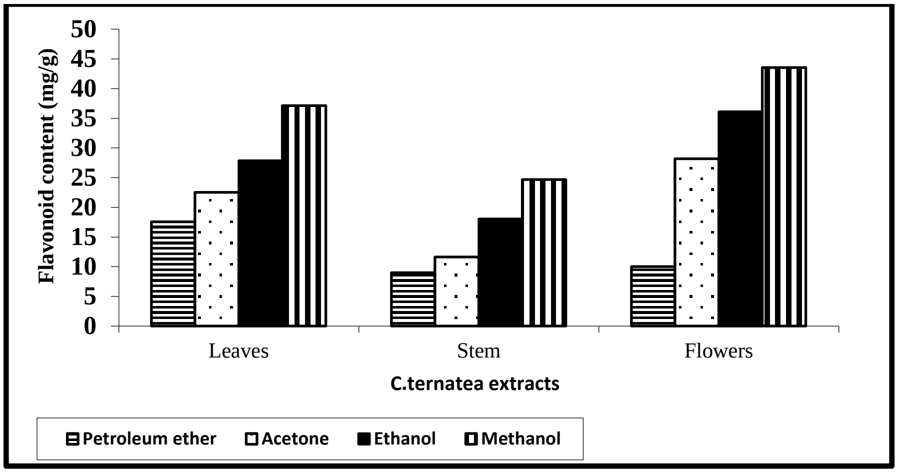

Figure.2. Total Flavonoid content of *C.ternatea* plant parts

Estimation of Quercetin by HPLC

Exact pattern of flavonoids in each extracts may be characterized either by HPLC or GC-MS. Crude extracts cannot be used for GC-MS analysis, it require even partial purification of the sample. But in the case of HPLC, crude extracts can be diluted and its fingerprinting pattern of flavonoids can be analyzed using specific wavelength. Chromatographic pattern of *C.ternatea* plant parts require first optimization of HPLC method, in this study mobile phase was standardized as methanol and 0.5% phosphoric acid and quercetin was used as standard (Retention time (Rt)= 3.2min). Previous report on HPLC analysis reported that retention time of quercetin from flower samples[6] was >20 min, but in this study quercetin was detected within 5mins of separation. Leaf, stem and flower extracts of *C.ternatea* showed presence of quercetin and other flavonoids, Based on peak area, concentration, and Rt, quercetin level in each extract was calculated and presented in Table.1. Flower extracts contain significantly higher quercetin content (298.32µg/g) than leaf (141.02µg/g) and stem extracts (108.18µg/g) and these results are in correlation with previous studies on *C.ternatea* petal extracts on flavonoid composition[7].

Table.1. HPLC Fingerprinting pattern of flavonoids (Rt, Peak area) Quercetin in plant parts of *C.ternatea*

Samples	Retention time (Rt)	Peak area [mV.s]	Flavonoids	Quercetin (mg/g)
Quercetin standard	3.078	20.146		100.00
C.ternatea extracts				
Leaves	2.061	10.115	UF	-
	3.070	23.812	Quercetin	141.02
	3.814	18.323	UF	-
	4.106	26.564	UF	-
	4.725	32.242	UF	-
Stem	2.120	17.468	UF	-

	2.855	12.341	UF	-
	3.074	18.125	Quercetin	108.18
	3.227	28.735	UF	-
	3.760	36.676	UF	-
	4.846	20.112	UF	-
Flowers	2.247	34.432	UF	-
	3.075	38.512	Quercetin	298.32
	3.663	26.238	UF	-
	4.216	21.725	UF	-

*UF- Unknown Flavonoid

Antidermatophytic acitivity

Different parts of *C.ternatea* methanolic extracts at three different concentrations were tested for their antifungal potential (Table.2.). Zone of inhibition was observed, which is ranged between 3.1 to 10.1mm. Pure standards, quercetin and ketoconazole showed highest zone of inhibition (8.2 to 16.8mm and 14.2 to 24mm respectively), surprisingly *C.ternatea* flower extracts (200µg/ml) also showed strongest inhibition (4.2 to 10.1mm) as compared to other extracts. Lower concentrations of plant extracts were not significantly effective on dermatophytes (Results not shown). But higher concentrations of stem extracts showed lowest inhibition (3.1 to 8.0mm), while leaf extracts exhibited significantly moderate inhibition (3.7 to 8.8mm) on fungal growth. During MFC testing, most of these extracts have partial inhibition (Table.3.) on dermatophytes at lower concentration (0.18-0.60mg/ml). Flower extracts inhibited all the fungal species tested at the concentration of <0.30mg/ml except *Trichophyton metagraphytes*, *Epidermophyton floccosum* and *Trichophyton rubrum*, which are inhibited at higher concentration of 0.5mg/ml. In case of stem and leaf extracts higher concentrations (>0.75mg/ml) are needed for strong inhibition of dermatophytes but lower concentrations are enough for controlling other fungi tested. Similarly, stem extracts has previously been reported to have antimicrobial activity at higher concentrations against *Rhizopus* and *Pencillium spp*[20]. In this study, *Aspergillus sp.*. is most susceptible fungi towards all the extracts and *T.metagraphytes* showed resistance at minimum concentration of extracts. Results of both disc diffusion method and MFC assay for antifungal activity are comparable, which showed flower extracts of *C.ternatea* is the strongest antidermatophytic drug and also have antimicrobial activity towards *Candida sp..*, *Fusarium sp.*. and *Aspergillus sp.* The results of this study suggest that quercetin and flavonoid in flower and leaf extracts may influence the antimicrobial activity and it might be due to the inhibition of nucleic acid biosynthesis in fungi by quercetin. Also, binding of quercetin to the membrane surface may be the cause of alteration in membrane formation and function in fungal species or by changing membrane potential on surface of fungal species in turn cause death of the fungal cells. And this is the first report on application of leaf, stem, and flower parts of *C.ternatea* against dermatophytes, and flower extracts may be a source of antimicrobial drug in future.

Table.2. Antidermatophytic activity of standards and the extracts of *Clitoria ternatea* on Zone of Inhibition (mm)

Fungi	**Zone of Inhibition (mm)**				
	Quercetin (30µg/ml)	**Ketaconazole (25µg/ml)**	**Leaves (200µg/ml)**	**Stem (200µg/ml)**	**Flower (200µg/ml)**
Aspergillus niger	16.3	26.2	8.8	8.0	10.1
Aspergillus flavus	14.5	24.8	8.3	7.2	9.4
Aspergillus fumigates	13.2	22.7	7.8	6.6	8.6
Candida albicans	16.0	28.0	8.0	6.9	9.0
Candida utilis	15.6	25.1	7.1	5.8	8.2
Fusarium oxysporum	12.8	20.2	7.4	6.4	8.0
Fusarium solani	13.0	21.6	7.7	7.0	9.5
Microsporum gypseum	10.6	18.7	6.1	5.6	7.6
Trichophyton metagraphytes	8.2	15.0	3.7	3.1	4.2
Epidermophyton floccosum	8.8	14.7	5.4	4.6	6.2
Trichophyton rubrum	9.0	14.2	5.0	4.0	4.8

Values are mean ±SD n=6 in each group

Table.3. Minimum Fungicidal Concentration (MFC) of *Clitoria ternatea* extracts

Fungi	**Minimum Fungicidal Concentration (mg/ml)**				
	Quercetin	**Leaves**	**Stem**	**Flower**	**Ketaconazole**
Aspergillus niger	0.12	0.25	0.25	0.18	0.01
Aspergillus flavus	0.12	0.25	0.25	0.20	0.05
Aspergillus fumigatus	0.17	0.35	0.45	0.25	0.03
Candida albicans	0.10	0.25	0.25	0.21	0.02
Candida utilis	0.14	0.25	0.25	0.20	0.02
Fusarium oxysporum	0.12	0.40	0.60	0.30	0.04
Fusarium solani	0.09	0.50	0.25	0.25	0.03
Microsporum gypseum	0.13	0.85	1.12	0.25	0.02
Trichophyton metagraphytes	0.18	1.12	1.25	0.50	0.03
Epidermophyton floccosum	0.16	0.50	0.75	0.35	0.02
Trichophyton rubrum	0.16	0.70	1.00	0.48	0.03

Values are mean ±SD n=6 in each group

CONCLUSION

Screening of *C.ternatea* plant tissues might lead to the novel identification flavonoids that are efficiently potent to be useful as antidermatophytic compounds. In addition, apart from quercetin and its derivatives, investigation of other compounds responsible for therapeutic use of plant is likely to be a future area of research. Also, further study of mechanism behind the resistance or susceptibility of dermatophytes towards flavonoids, mechanism of spreading using macro/micro conidia and its decrease in growth on skin after flavonoid treatment could be useful for treatment of dermatophytosis.

REFERENCES

1. Bhatia M, Chahal J, Gupta JCS. Analgesic and anti-inflammatory activities of *Clitoria ternatea* Linn. leaves extract on rat model. Int J Pharm Sci Res 2014; *5*(2): 600-602.
2. Saxena AK, Vikas S, Pankaj M, Amit K. Comparative hypoglycemic effects of different extract of *Clitoria ternatea* leaves. Journal of pharmacology and toxicological studies 2014; *2*(3): 25-30.
3. Solanki YB, Jain SM. Hepatoprotective effects of *Clitoria ternatea* and *Vigna mungo* against acetaminophen and carbon tetrachloride-induced hepatotoxicity in rats. J Pharmacol Toxicol 2011; 6: 30-48.
4. Eftekhari M, Alizadeh M, Ebrahimi P. Evaluation of the total phenolics and quercetin content of foliage in mycorrhizal grape (*Vitis vinifera* L.) varieties and effect of postharvest drying on quercetin yield. Ind Crops Prod 2012; 38: 160-165.
5. Swain SS, Rout KK, Chand PK. Production of triterpenoid anti-cancer compound taraxerol in Agrobacterium-transformed root cultures of butterfly pea (*Clitoria ternatea* L.). Appl Biochem Biotechnol 2012; 168(3): 487-503.
6. Kazuma K, Noda N, Suzuki M. Malonylated flavonol glycosides from the petals of *Clitoria ternatea*. Phytochemistry 2003; 62(2): 229-237.
7. Kazuma K, Noda N, Suzuki M. Flavonoid composition related to petal color in different lines of *Clitoria ternatea*. Phytochemistry 2003; 64(6) 1133-1139.
8. Phrueksanan W, Yibchok-anun S, Adisakwattana S. Protection of *Clitoria ternatea* flower petal extract against free radical-induced hemolysis and oxidative damage in canine erythrocytes. Res Vet Sci 2014; DOI: 10.1016/j.rvsc.2014.08.010.
9. Daisy P, Santosh K, Rajathi M. Antihyperglycemic and antihyperlipidemic effects of *Clitoria ternatea* Linn. in alloxan-induced diabetic rats. Afr J Microbiol Res 2009; 3(5): 287-291.
10. Grumbt M, Monod M, Yamada T, Hertweck C, Kunert J, Staib P. Keratin degradation by dermatophytes relies on cysteine dioxygenase and a sulfite efflux pump. J Invest Dermatol 2013; 133(6): 1550-1555.

11. Baldo A, Monod M, Mathy A, Cambier L, Bagut ET, Defaweux V, Symoens F, Antonie N, Mignon, B. Mechanisms of skin adherence and invasion by dermatophytes. Mycoses 2012; 55(3): 218-223.
12. Cronin LJ, Mildren RP, Moffitt M, Lauto A, Morton CO, Stack CM. An investigation into the inhibitory effect of ultraviolet radiation on *Trichophyton rubrum*. Lasers Med Sci, 2014; 29(1): 157-163.
13. Patankar M, Bhargava A, Ahluwalia A. Comparison of the minimum fungicidal concentration of clotrimazole, ketoconazole, miconazole and terbinafine against clinical isolates of dermatophytes. Indian Medical Gazette, 2014; CXLVII(2): 65-68.
14. Dixit PK, Mittal S. An overview of herbal antimicrobial agents: Boon for treatment of microbial infections. Int J Pharm Res Dev 2013; 5(6): 38-45.
15. Kelemu S, Cardona C, Segura G. Antimicrobial and insecticidal protein isolated from seeds of *Clitoria ternatea*, a tropical forage legume. Plant Physiol Bioch 2004; 42(11): 867-873.
16. Kamilla L, Mansor SM, Ramanathan S, Sasidharan S. Effects of *Clitoria ternatea* leaf extract on growth and morphogenesis of *Aspergillus niger*. Microsc Microanal 2009; 15(04): 366-372.
17. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 2002; 10(3): 178-182.
18. Sachan D, Singh, JP, Sachan D, Gangwar S. Design, synthesis and antimicrobial evaluation of mannich bases of novel isoindole analogues. Am J Pharmtech Res 2012; 2(4): 492-497.
19. Cushnie TP, Lamb AJ. Recent advances in understanding the antibacterial properties of flavonoids. Int J Antimicrob agents 2011; *38*(2): 99-107.
20. Kamilla L, Mnsor SM, Ramanathan S, Sasidharan S. Antimicrobial activity of *Clitoria ternatea* (L.) extracts. Pharmacologyonline 2009; 1: 731-738.