ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 6, Issue 4, 2013

ISSN - 0974-2441

Research Article

PHYTOCHEMICAL CHARACTERIZATION OF Toddalia asiatica.L Var. Floribunda STEM

PRAVEENA,A[1], SURIYAVATHANA,M[2]*

[1]Department of Biochemistry, KSR College of Arts and Science, Tiruchengode, Namakkal DT. [2]*Department of Biochemistry, Periyar University, Salem-11. Email: praveenaarun78@gmail.com

Received: 29 August 2013, Revised and Accepted: 15 September 2013

ABSTRACT

Objective: Plants been used in traditional medicine for several thousand years. India is a home to a variety of traditional medicine systems that relay to a very large extent on native plant species for their raw drug materials. There are many reports on the use of plants in the traditional healing by either tribal people or indigenous communities of India. Now there is a need to look back towards the traditional medicine which can serve as novel therapeutics. *Toddalia asiatica*, has been in folklore use in India and China from 18th century. Since, this plant possess many medicinal properties, the present study was designed to evaluate the phytochemicals of stem extract of *Toddalia asiatica.L.* Var Floribunda. Methods: The phytochemicals of stem extract of *Toddalia asiatica.L.* Var Floribunda was analysed qualitatively and the presence of some phytochemicals are confirmed by HPTLC and GC/MS analysis. Results:The results of the above study conclusively validate the phytochemical treasures indulged in *Toddalia asiatica.* Var Floribunda

Keywords: Toddalia asiatica. L Var Floribunda, methanolic extract, phytochemicals, alkaloids, coumarins, HPTLC and GC-MS.

INTRODUCTION

Medicinal plants acts as a raw material base for the elaboration of more complex semi-synthetic chemical compounds. Many of these isolations from the medicinal plants were based on the uses of the agents in traditional medicine. During the last few decades there has been an increasing interest in the study of medicinal plants and their traditional use. There has been a resurgence in the consumption and demand of medicinal plants. Traditional medical knowledge of medicinal plants and their use by indigenous cultures are not only useful for the conservation of cultural traditions and biodiversity but also for community healthcare and drug development in the future. Now there is a growing interest in correlating each phytochemical constituent of a plant with its pharmacological activity[1]. The biologically active compounds like alkaloids, flavonoids, tannins and phenolic compounds are the main reason for the medicinal value of plants that produce a definite physiological action on the body if it is administered [2].

MATERIALS AND METHODS

Plant materials

The plant material (stem) of *Toddalia asiatica* Var Floribunda was collected at Kolli hills, Namakkal District which is rich in wide variety of medicinal plants. The collected sample was identified and confirmed by BSI, Coimbatore. The stem part was air dried and powdered.

Preparation of the extract for phytochemical analysis

10 grams pulverized material were dissolved in 100 ml of solvent of methanol and kept in a shaker for overnight. The obtained extracts were filtered with Whatmann No.4 filter paper and the filtrate was collected and used for qualitative analysis of phytochemicals like Alkaloids, Flavonoids, Saponins, Steroids, Glycosides, Tannins, carbohydrates, proteins and aminoacids[3].

HPTLC Analysis

The methanolic extract of the stem was then analysed for the confirmation of the phytochemicals like coumarins and alkaloids.

The dried methanolic extract 100mg was weighed in an electronic balance (Afcoset) accurately and dissolved in 1ml of Methanol and centrifuged at 3000rpm for 5min. These solutions were used as test solution for HPTLC analysis. 5µl of standard nicotine solution and 2µl of the above test solutions were loaded as 5mm band length in the 6 x 10cm Silica gel 60F_{254} TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase Chloroform-methanol (8 : 4) (Alkaloid) and the plate was developed in the respective mobile phase up to 90mm. with respective mobile phase, and for coumarin Toluene-ether (1 : 1) saturated with 10% acetic acid (Coumarin) and the plate was developed in the respective mobile phase up to 90mm.

The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at White light, UV 254nm and UV366nm.

The developed plate was sprayed with respective spray reagent Dragendorff's reagent followed by 10% Ethanolic Sulphuric acid reagent for Alkaloid and dried at 100°C in Hot air oven. For coumarin the developed plate was sprayed with respective spray reagent, 10% Ethanolic Potassium hydroxide reagent (Coumarin) and dried at 100°C in Hot air oven.

The plate was photo-documented at Day light mode using Photo-documentation (CAMAG REPROSTAR 3) chamber. After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at 500nm. The Peak table, Peak display and Peak densitogram were noted.

GC/MS Analysis

The methanolic extract of *Toddalia asiatica* Var Floribunda stem was analysed by GC-MS (THERMO GC - TRACE ULTRA VER: 5.0, THERMO MS DSQ II). The GC column dimension used was 30 x.25mm x .25mm, fused with TR 5 - MS capillary standard non - polar column. The temperature was programmed from 80° to 250° C at the increase in rate of 8° C per minute Helium gas was engaged as a carrier gas at the rate of 1.0 ml/ min. The spectra were obtained in the eI mode with 70eV ionization energy. Mass spectra of the separated components from the extracts were compared with the known components. The name, molecular weight and structure and the activity of the components were determined.

RESULTS

The qualitative phytochemical analysis reveals that the methanolic stem extract of *Toddalia asiatica.L.* Var Floribunda contains secondary metabolites like Alkaloids, Flavonoids, Saponins, Steroids, Glycosides, Tannins (Table 1) and the presence of coumarins and alkaloids were confirmed by HPTLC which reveals the presence of 3 different alkaloids (Table 2) and 7 different coumarins(Table 3).

Table 1: Phytochemical (Qualitative) analysis of stem extract of *Toddalia asiatica.L* Var Floribunda

S.No.	Phytochemicals	Tests	Result
1.	Carbohydrates	Molisch test Fehling's test	+
2.	Proteins & Amino acids	Ninhydrin Test Biuret Test	+
3.	Flavonoids	Alkaline Reaction (Color with NaOH) Shinoda test	+
4.	Alkaloids	Mayer's Test Dragondroff's Test	+
5.	Tannins,phenols	Ferric chloride Test	+
6.	Steroids, Phytosterols	Libermann burchard	+
7.	Saponins	Foam test	+
8.	Glycosides	Legals Test	+
12	Coumarins	Alkaline Test	+

Table 2: HPTLC Peak table for alkaloid

S. No	Track	Peak	Rf	Height	Area	Assigned substance
1	NIC	1	0.67	300.2	9106.9	Nicotine standard
2	Sample B	1	0.01	163.3	3103.9	Unknown
3	Sample B	2	0.08	16.7	147.7	Unknown
4	Sample B	3	0.1	34.1	530.4	Unknown
5	Sample B	4	0.2	61.9	2411.9	Alkaloid 1
6	Sample B	5	0.22	64.3	2141.5	Unknown
7	Sample B	6	0.37	31	742.6	Alkaloid 2
8	Sample B	7	0.61	28	755.4	Unknown
9	Sample B	8	0.76	20.5	450.2	Unknown
10	Sample B	9	0.82	32.2	658.7	Unknown
11	Sample B	10	0.84	36.2	1055.6	Alkaloid 3
12	Sample B	11	0.93	215.4	5411.1	Unknown
13	Sample B	12	0.97	70.4	755.3	Unknown

In GC-Ms analysis, the active compounds identified in the methanolic extract were represented as gas chromatogram (Figure 1) and its mass spectrum (Figure 4). Totally twenty five compounds have been detected through GC-MS analysis based on retention time, molecular formula, molecular weight and peak area.. The active compounds with their retention time (RT), molecular formula, molecular weight (MW) and concentration (peak area %) are presented in Table-4. The major compounds present were 2,3-diphenyl-4-acetoamidothiophene (39.80 %), 2-(4-Pyridyl)-4-methylquinoline (19.14%) and Pyrido[2,3-b]indole (16.48%) and other major and minor compounds were also present. GC/MS analysis also shows the presence of many important compounds (Table 4) with 5 different alkaloids and 2 different coumarins.

Table 3: HPTLC Peak table for coumarin

S.No	Track	Peak	Rf	Height	Area	Assigned substance
1	COU	1	0.66	311.5	15367.2	Coumarin standard
2	Sample B	1	0.04	25.6	236.3	Unknown
3	Sample B	2	0.09	254.2	4417.6	Coumarin 1
4	Sample B	3	0.17	29.5	330.2	Unknown
5	Sample B	4	0.30	89.3	1703.6	Coumarin 2
6	Sample B	5	0.37	69.7	1313.0	Coumarin 3
7	Sample B	6	0.39	77.1	780.0	Unknown
8	Sample B	7	0.44	67.1	1727.6	Coumarin 4
9	Sample B	8	0.52	171.5	6299.9	Coumarin 5
10	Sample B	9	0.70	156.6	3422.9	Coumarin 6
11	Sample B	10	0.71	162.6	2991.7	Unknown
12	Sample B	11	0.86	105.7	3470.6	Coumarin 7

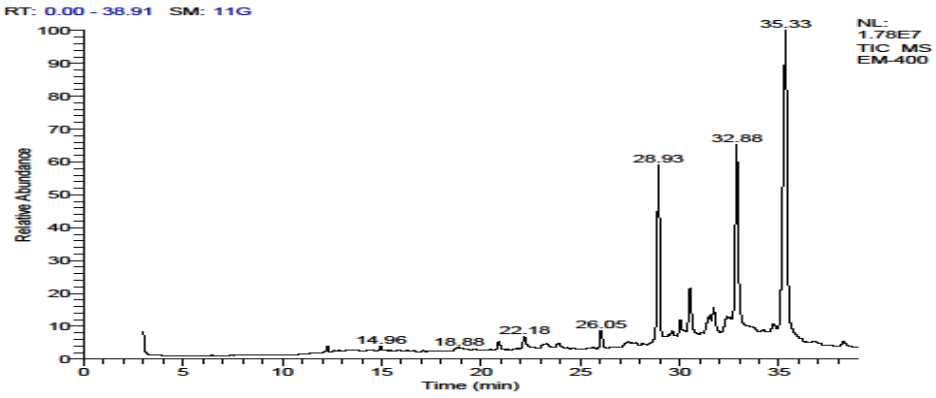

Figure 1: Gas Chromatogram of methanolic extract of *Toddalia asiatica*

Table 4: The activity of the various Phytocomponents identified in the sample extract by GC-MS

S. No	RT	Name of the compound	Molecular Formula	MW	Peak area %	Compound Nature	Activity
1	12.27	trans-Caryophyllene	$C_{15}H_{24}$	204	0.56	Sesquiterpene	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory Sedative, Fungicide
2	12.60	5-Hydroxy-6-methyl-3-oxo-4-[[(6-methoxypyridin-3-yl)methylene]amino]-2,3,4,5-tetrahydro-1,2,4-triazine	$C_{11}H_{13}N_5O_3$	263	0.19	Amino compound	Antimicrobial
3	12.86	7-anti[(exo)-Bicyclo[2.1.0]pent-5-yl]-2,3-diazabicyclo[2.2.1]hept-2-ene	$C_{10}H_{14}N_2$	162	0.18	Nitrogen compound	Antimicrobial
4	14.96	E-Dodec-3-en-5-yn-1-ol	$C_{12}H_{20}O$	180	0.41	Unsaturated alcohol	Antimicrobial
5	15.92	{2-[Bromomethyl]-3,4-dihydro-2H-pyrrol-5-yl]pyrrolidin-2-ylidene}ac Etonitrile	$C_{11}H_{14}BrN_3$	267	0.16	Alkaloid	Antimicrobial Anti-inflammatory
6	18.86	Benzyl 2-Iodobenzyl Sulfide	$C_{14}H_{13}IS$	340	0.59	Sulphur compound	Antimicrobial
7	20.89	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.67	Palmitic acid ester	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor
8	22.16	á-D-Glucopyranoside, methyl 2,4,6-tri-O-methyl	$C_{10}H_{20}O_6$	236	1.10	Sugar moiety	Preservative
9	23.32	Bis(octa-2,7-dienyl) ether	$C_{16}H_{26}O$	234	0.78	Ether compound	No activity reported
10	23.92	(M)-2-Aminomethyl-1-(2'-hydroxy-4',6'-dimethylphenyl)-5,6,7,8-tetrahydronaphthalene	$C_{19}H_{23}NO$	281	0.45	Naphthalene compound	Insecticide
11	26.05	N-Methylfindersine (2,2,6-Trimethyl-5,6-dihydro-2H-pyrano[3,2-c]quinoline-5-one)	$C_{15}H_{15}NO_2$	241	1.81	Alkaloid	Antimicrobial Anti-inflammatory
12	27.38	3-(4-Methyl-1,3-cyclohexadienyl)butanoic acid	$C_{11}H_{16}O_2$	180	1.08	Acidic compound	Antimicrobial
13	28.93	Pyrido[2,3-b]indole	$C_{18}H_{14}N_2O$	274	16.48	Alkaloid	Antimicrobial Anti-inflammatory
14	29.62	Pentadecanal	$C_{15}H_{30}O$	226	0.89	Aldehyde	Antimicrobial
15	30.02	3-Hydroxymethyl-4-(1',2',4'-triazol-5'-yl)thioquinaldine	$C_{13}H_{12}N_4OS$	272	1.51	Alkaloid	Antimicrobial Anti-inflammatory
16	30.52	5,7-Dimethoxy-8-(2'-keto-3-methylbutyl) coumarin	$C_{16}H_{18}O_5$	290	4.83	Coumarin compound	Antimicrobial Anticancer Antiinflammatory Hypoglycemic Fungicide Antitumor Analgesic Hepatotoxic Sedative Pesticide Rodenticide Chemopreventive
17	31.70	Sibirinol	$C_{16}H_{18}O_5$	290	5.55	Aromatic alcohol	Antimicrobial Antioxidant Anti-inflammatory
18	32.28	4-(7-oxo-1,3,4,5,6,7-hexahydro-2H-azepin-1-yl)-4,7-dihydro-2,9-benzo Furandione	$C_{14}H_{17}NO_4$	263	1.12	Furan compound	No activity reported
19	32.88	2-(4-Pyridyl)-4-methylquinoline	$C_{15}H_{12}N_2$	220	19.14	Alkaloid	Antimicrobial Anti-inflammatory
20	33.71	1-methylethyl propanoate	$C_6H_{12}O_2$	116	0.61	Ester compound	No activity reported

21	34.22	rac-4,4'-Dimethyl-4,4'-bi-3,4-dihydrocoumarin	$C_{20}H_{18}O_4$	322	0.25	Coumarin compound	Antimicrobial Anticancer Antiinflammatory Hypoglycemic Fungicide Antitumor Analgesic Hepatotoxic Sedative Pesticide Rodenticide Chemopreventive
22	34.74	7-Phenyl-5H-thiazolo[5,4-e]pyrrolo[1,2-a]-(1,4)-diazepin-10(9H)-one	$C_{15}H_{11}N_3OS$	281	0.90	Sulfur compound	Antimicrobial
23	35.53	2,3-diphenyl-4-acetoamidothiophene	$C_{18}H_{15}NOS$	293	39.80	Sulfur compound	Antimicrobial
24	36.83	1,5-Cyclododecadiene, (Z,Z)	$C_{12}H_{20}$	164	0.31	Alkene compound	No activity reported
25	38.26	4,4-dimethyl-1,2,3-oxadithiane-2-oxide	$C_5H_{10}O_2S_2$	166	0.62	Sulfur compound	Antimicrobial

DISCUSSION

Plant produces these phytochemicals to protect themselves from bacteria and other predatorial invaders, but research has discovered that plants with phytochemical abilities may also protect humans from illness. In the present study the phytochemical screening of methanolic stem extract shows the presence of some phytochemicals like Alkaloids, Flavonoids, Saponins, Steroids, Glycosides, Tannins and the presence of coumarins and alkaloids were confirmed by HPTLC are confirmed by HPTLC and GC/MS analysis.

Alkaloids are one of the diverse groups of secondary metabolites which found to have antimicrobial activity by inhibiting DNA topoisomerase in the microorganisms [4]. Flavanoids are phenolic compounds and acts as natural biological modifiers. There exists a direct relationship between the levels of phenolic compounds and antioxidant potential of plants [5]. Phenolic compounds exhibit their protective action through various mechanisms like preventing the generation of carcinogens from precursors by acting as blocking agents [6]. Coumarin has antifungal and anticancer activity. Coumarin increases the blood flow in the veins and decreases capillary permeability. Secondary metabolite studies have shown that the presences of carbohydrate, flavanoid, alkaloid, tannin, coumarin, steroid, phenol are of great importance in the field of ayurvedic drug research. The compound rich in phenolic content can reduce the risk of heart disease by slowing the progression of atherosclerosis by acting as antioxidants towards LDL. All these studies will be of immense use in carrying out further research and revalidation of its use in Ayurvedic system of medicine. Similar studies on the phytochemicals of the leaf was done and analysed by GC-MS analysis which reveals many compounds and their activities[7].

CONCLUSION

From these studies, a conclusion can be drawn that *Toddalia asiatica* Stem can have more beneficial effects with respect to the presence of many active secondary metabolites which may likely to combating diseases like cancer, cardio-vascular diseases and in general boost the immunesystem. Further detailed studies can be carried out in order to isolate active components like alkaloid and coumarin responsible for particular activity. It is believed that information obtained would help to get aware of this plant and extensive research should be undertaken on this plant phytochemicals for estabilishing new therapeutic drugs for mankind.

ACKNOWLEDGEMENT

The authors are grateful to Department of Biochemistry, Periyar University, Salem-11 and KSR College of Arts and Science, Tiruchengode, Namakkal DT.

REFERENCES

1. Gupta, SS. 1994. Prospects and perspectives of natural plant products in medicine. Indian Journal of Pharmacol, 26:1-12.
2. Hemashenpagam, N., Lali Growther, Sankar, Selvaraj, T. and Panneerselvam, A. 2009. Photochemical analysis and antimicrobial activity of *Solanum xanthocarpum.* Biomedicine, 29 (4): 353-356.
3. Kokate, CK. 1994. Practical Pharmacognosy, Vallabhi Prasashan Kakatiya University, Warangal, A.P. India 109-114.
4. Bonjean, K., De Pauw-Gillet M-C, *et al.,* 1998, J. Ethnopharmacol 69, 241-246.
5. Robards, K, Prenzler, PD., Tucker, G. 1999, Phenolic compounds and their role in oxidative processes in fruits, Food Chem, 66,401-436.
6. Wattenberg, LW and Lam, LKT. 1983, Phenolic antioxidants as protective agents in chemical carcinogenesis. In: Radioprotectors and Anticarcinogens , O. F. Nygaard, and M. G. Simic, Eds. Academic Press, New York, 461-469.
7. Pavithra Kalaisezhiyen and Vadivukkarasi Sasikumar. 2012, GC- MS Evaluation Of Chemical Constituents from Methanolic Leaf Extract of *Kedrostis foetidissima* (Jacq.) Cogn, Asian Journal of Pharmaceutical and Clinical Research, Vol 5, Suppl 4, 77-81.