Academic Sciences

International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 5, Issue 2, 2013

Research Article

EXTRACTION AND QUANTIFICATION OF STEROLS FROM *TRIBULUS TERRESTRIS* L., *SIDA ACUTA* BURM F. AND *TRIDAX PROCUMBENS* L.

ALKA JINDAL, PADMA KUMAR

Laboratory of Plant Tissue Culture and Secondary Metabolites, Department of Botany, University of Rajasthan, Jaipur 302044, Rajasthan, India. Email: jindal4@gmail.com

Received: 06 February 2013, Revised and Accepted: 31 March 2013

ABSTRACT

Extraction of secondary metabolites from medicinal plants has become important for the presence of bioactive compounds. The present study was aimed to detect and extract sterols in the different parts of *Tribulus terrestris* L. (root, stem, leaf and fruits), *Sida acuta* Burm f. (root, stem, leaf and buds) and *Tridax procumbens* L. (root, stem, leaf, bud, flower and pedicle). Preliminary detection and extracts of sterols was performed by well established methods. The extracts were separately dried and weighed. Result revealed that all the parts of the selected plants were rich in sterol content. Maximum sterol content recorded were 50.5, 34.7 and 18.6 mg/g.d.w for *T. terrestris*, *S. acuta* and *T. procumbens*, respectively.

Keywords: Sterols, *Tribulus terrestris*, *Sida acuta, Tridax procumbens*

INTRODUCTION

Medicinal plants are essential part of human medicine, since the dawn of civilization and are the backbone of traditional medicine system in India [1]. They represent rich source of antimicrobial agents [2]. Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper rate than the modern medicine [3]. Many plant families represent reservoir of effective chemotheraupeutics and can provide valuable sources of natural antimicrobials [4,5].

T. terrestris (Family: Zygophyllaceae) is an annual plant distributed in warm regions of Asia, Africa, Europe, America and Australia [6-8]. It is an important medicinal plant and has been used extensively as tonic, aphrodisiac, analgesic, astringent, stomachic, anti-hypertensive, diuretic, lithontriptic and urinary anti-infective [9]. The main constituents of plant are saponins, diosgenins, alkaloids and amides [10-12].

S. acuta (Family: Malvaceae) is an erect perennial shrub found throughout the hotter parts of India and Nepal. The plant has been used for eczema, kidney stone, headache, malaria, ulcer, fever, gonorrhea, abortion, breast cancer, poisoning, inflammation, feed for livestock, stops bleeding, treatment of sores, wounds and antipyretic [13-16].

T. procumbens (Family: Asteraceae) is a perennial herb. The plant has been used as feed for livestock and stops bleeding [17,18] and for treatment of diarrhea, malaria, cough and asthma, boils, epilepsy, liquid purging, wounds, toothache and stomachache and paralysis [19-20].

MATERIAL AND METHODS

Plant Material

Different parts of *Tribulus terrestris* (root, stem, leaf and fruits), *Sida acuta* (root, stem, leaf and buds) and *Tridax procumbens* (root, stem, leaf, bud, flower and pedicle) were collected from different localities of Jaipur, in the month of June, 2008. Selected plants were identified at Herbarium, Department of Botany, University of Rajasthan, Jaipur. Voucher specimens (RUBL-20390, 20428 and 20389, respectively) were also submitted to the Herbarium, UOR. All the parts of selected plants were separately shade dried and were milled to a fine powder using a grinder.

Detection of Sterols

Two standard methods were used to determine the presence of sterols in each sample of selected plants [21,22]. 20 grams of finely powdered samples were Soxhlet extracted with hot methanol (200 ml) on a water bath for 24 h and filtered by using Whatman filter paper No. 1. The extracts were concentrated on water bath (for removal of excess methanol) and were used for the detection of sterols.

Liberman-Burchard's Test: 5 ml of chloroform and 2 ml of acetic acid were added to a portion of the methanol extract of each part of the selected plants. Appearance of green colour in each extract, indicate the presence of sterols.

Salkowski Test: Few drops of con. H_2SO_4 solution was added to each test extracts. A purple colour ring was observed at the upper surface, showed the presence of sterols.

Extraction and Quantification of Sterols

Different parts of selected plants viz., *T. terrestris* (root, stem, leaves and fruits), *Sida acuta* (root, stem, leaves and buds) and *T. procumbens* (root, stem, leaves, bud, flower and pedicle) were subjected to the sterols extraction [23]. Hundred grams of finely powdered plant part was Soxhlet extracted with hot petroleum ether (500 ml) on a water bath for 24 h and filtered. Residual mass is taken and hydrolyzed in 30% HCl for 4 h on water bath. Resulting mixture was washed with distilled water till neutrality and dried in oven at 50°C. The dried material was dissolved in benzene for 1 day and filtered. Filtrates were dried in *vaccuo*, weighed and stored in glass vials at 4°C till used.

Table 1: Detection of sterols of *T. terrestris*, *S. acuta* and *T. procumbens*

Plant Part	*T. terrestris*		*Sida acuta*		*T. procumbens*	
	Burchard's	Solkowski	Burchard's	Solkowski	Burchard's	Solkowski
Root	++	-	-	++	-	+
Stem	++	+	++	-	+	-
Leaf	+++	++	+	-	++	+
Fruit	-	++	Nd	Nd	Nd	Nd
Bud	Nd	Nd	+	+	+++	+
Flower	Nd	Nd	Nd	Nd	+	++
Pedicle	Nd	Nd	Nd	Nd	-	+

- : absent; +: trace amount; ++: moderate amount; +++: significant amount; Nd: not determined

Table 2: Quantitative estimation of sterols of *T. terrestris*, *S. acuta* and *T. procumbens*

Plant part	Sterols (mg/g.d.w)		
	T. terrestris	***Sida acuta***	***T. procumbens***
Root	24	22	7
Stem	28.45	18.2	4.7
Leaf	50.5	11.8	15
Fruit	15	Nd	Nd
Bud	Nd	34.7	9.2
Flower	Nd	Nd	18.6
Pedicle	Nd	Nd	9.5

Nd: Not determined

RESULTS

In the present investigation preliminary detection of sterols has been done in the methanol extract of different parts of *T. terrestris*, *S. acuta* and *T. procumbens* and the results are presented in Table 1. All the methanol extract of the selected plants showed presence of sterols by showing positive response for Burchard's and/or Salkowaski test.

Sterol content estimated in each gram of dried plant material of *T. terrestris*, *S. acuta* and *T. procumbens* was recorded in Table 2. Content of sterols were obtained maximum in leaf of *T. terrestris* (50.5 mg/g.d.w), followed by bud of *S. acuta* (34.7 mg/g.d.w) and flower of *T. procumbens* (18.6 mg/g.d.w).

DISCUSSION

Sterols are subgroup of the steroids and occur naturally in plants, animals and fungi. They cannot be synthesized by humans and are thus consumed from the diet. They are incorporated in a variety of food products [24]due to their cholesterol-lowering effect, hence providing protection against cardiovascular disease [25]. They have shown inhibition of several cancer cell lines including colon [26], prostate [27] and breast [28]. The role of plant sterols as immune modulators [29] and anti-inflammatory agents [30]has also been described. Hence, it is need to screen medicinal plants for detection and extraction of sterols. Result revealed that the selected three plants (*T. terrestris*, *S. acuta* and *T. procumbens*) can be exploited as an important source of phytosterol for drug formulations.

ACKNOWLEDGEMENT

Authors are thankful to the Head of Botany Department, University of Rajasthan, Jaipur, India for providing all necessary facilities to carry out the work. Financial assistance provided by UGC is gratefully acknowledged.

REFERENCES

1. Nayak A, Nayak RN, Soumya B, Bhat K and Kudalkar M. 2011. Evaluation of antibacterial and anticandidal efficacy of aqueous and alcoholic extracts of Neem (*Azadirachia India*) and *in vitro* study. IJPAP. 1: 230-235.
2. Mahesh B and Satish S. 2008. Antimicrobial activity of some important medicinal plant against plant and human pathogens. World J Agri Sci. 4 (S): 839-843.
3. Mann A, Banso A and Clifford LC. 2008. An antifungal property of crude plant extracts from *Anogeissus leiocarpus* and *Terminalia avicennioides*. Tanzania J Health Res. 10(1): 34-38.
4. Balandrin MF, Klocke JA, Wutule ES and Bollinger WH. 1985. Natural plant chemicals: Sources of industrial and medicinal materials. Science. 228: 1154-1160.
5. Satish S, Raveesha KA and Janardhana GR. 1999. Antibacterial activity of plant extracts of phytopathogenic *Xanthomonas campestris* pathovars. Letters of appl Microbiol. 28: 145-147.
6. Topia MO, Giordano MA, Gueper HG. An Outbreak of Hepatogenous Photosensitization in Sheep Grazing Tribulus terrestris in Argentina. *Vet Hum Toxicol* 1994; **36(4):**311-3.
7. Abeywickrama K, Bean GA. Toxigenic Aspergillus Flavus and Aflatoxins in Sri Lankan Medicinal Plant Material. *Mycopathologia* 1991; **113:187**-90.
8. Kostova I, Dinchev D, Rentsch GH, Dimitrov V, Ivanova A. Two New Sulfated Furostanol Saponins from Tribulus terrestris. *Z Naturforsch [C]* 2002;57(1-2):33-8.
9. Ody P. The Complete Guide Medicinal Herbal. London: Dorling Kindersley; 2000. p. 223.
10. Evans WC. Trease and Evans Pharmacognosy. 15th ed. Edinburgh: W.B. Saunders; 2002. p. 481.
11. Yan W, Ohtani K, Kasai R, Yamasaki K. Steroidal Saponins from Fruits of Tribulus terrestris. *Phytochemistry* 1996;**42:**1417-22.
12. Borke CA, Stevens GR, Garriqan MJ. Locomotor Effects in Sheeps of Alkaloids Identified in Australian Tribulus terrestris. *Aust Vet J* 1992;**69:**163-5.
13. Anani K, Hudson JB, De Souza C, Akpkagana K, Tower GHN, Amason JT and Gbeassor M. 2000. Investigation of medicinal plants of Togo for antiviral and antimicrobial activities. Pharma Biol. 38: 40-45.
14. Kayode J. 2006. Conservation of indigenous medicinal botanicals in Ekiti State, Nigeria. J Zhejiang University Sci B. 7: 713-718.
15. Edeoga HO, DE Okwu and BO Mbaebie. 2005. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotech. 4: 685-688.
16. Sanganuwan AS and Gulumbe ML. 2006. Evaluation of *Sida acuta* subspecie *acuta* leaf/flower combination for antimicrobial activity and phytochemical constituents. Afr J Clin Exp Microbiol. 7: 83-88.
17. Egunjiobi JK. 1969. Some common weeds of West Africa. Bull. Res. Div. Ministry of Agric. Natural Resources Western State, Ibadan, Nigeria.
18. Holm L, Del Y, Holm E, Panchon T and Herberger T. 1997. World weeds natural histories and distributions. John Wiley and sons Inc, NewYork.
19. Udupa AL, Kulkarni DR and Udupa SL. 1995. Effect of *Tridax procumbens* extracts on wound healing. Int J Pharmacog. 33: 37-40.
20. Singh VK, Govil JN, Hashmi S and Singh G. 2003. Ethnomedicine and pharmacognosy- II, Studium Press. USA. 7. pp. 200.
21. Burchard H. 1889. Beitrage zur kenntnis des cholesterins. Inaugural-dissertation, Universitat Rostock.Chem. Zentralbl. 61(I): 25–27.
22. Salkowski E. 1872. Ber dtsch chem Ges. 5: 637.
23. Kaul B and Staba EJ. 1968. *Dioscorea* Tissue Cultures. I. Biosynthesis and isolation of diosgenin from *Dioscorea deltoidea* callus and suspension cells. Lloydia. 31(2): 171-179.
24. Vorster HH, Raal FJ, Ubbink JB, Marais AD, Rajput MC and Ntanois FY. 2003. Functional foods with added plant sterols for treatment of hypercholesterolaemia and prevention of ischaemic heart disease. SAJCN. 16: 49-58.
25. Tapiero H, Townsend DM and Tew KD. 2003. Phytosterols in the prevention of human pathologies. Biomed Pharmacother. 57: 321-325.
26. Choi YH, Kong KR, Kim YA, Jung KO, Kil JH, Rhee SH and Park KY. 2003. Induction of Bax and activation of caspases during β-sitosterol-mediated apoptosis in human colon cancer cells. Int J Oncol. 23: 1657-1662.
27. Von Holtz RL, Fink CS and Awad AB. 1998. β-sitosterol activates the sphingomyelin cycle and induces apoptosis in LNCaP human prostate cancer cells. Nutr Cancer. 32: 8-12.

28. Steenkamp V and Gouws MC. 2006. Cytotoxicity of six South African medicinal plant extracts used in the treatment of cancer. SAJB 72: 630-633.
29. Breytenbach U, Clark A, Lamprecht J and Bouic PJD. 2001. Flow cytometry analysis of the Th1-Th2 balance in healthy individuals and patients infected with the human immunodeficiency virus (HIV) receiving a plant sterol/sterolin mixture. Cell Biol Int. 25: 43-49.
30. Quilez J, Garcia-Lorda P and Salas-Salvado J. 2003. Potential uses and benefits of phytosterols in diet: present situation and future directions. Clin Nutr. 22: 343-351.