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## QUANTITATIVE ESTIMATION OF FUROSTANOL SAPONINS IN SELECTED MEDICINAL PLANTS OF NEPAL HAVING TRADITIONAL IMPORTANCE

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### ABSTRACT

Specific parts of highly medicinal plants including *Dioscorea deltoidea*, *Bacopa monierri*, *Asparagus racemosus*, *Withania somnifera*, *Gentiana lutea*, *Smilax sarsaparilla*, *Glycyrrhiza glabra*, *Tribulus terrestris*, *Allium cepa* and *Capsicum annum* are used as potent precursors in the traditional system of medicine for infertility, low libido and impotence in both men and women. Using chemical assay procedures, the active saponin fraction (furostanol) in the herbs were quantified through their selective extraction and purification. High furostanol saponin contents 5.26% was found in the *W. somnifera* and relatively low concentration of the furostanol concentration was found in *C. annum* (2.66%).

## BACKGROUND

Saponins are a large family of structurally-related compounds of steroid or triterpenoid aglycone (sapogenin) linked to one or more oligosaccharide moieties by glycosidic linkage. The carbohydrate moiety consists of pentoses, hexoses, or uronic acids. The presence of both polar (sugar) and nonpolar (steroid or triterpene) groups provide saponins with strong surface-active properties. Their physiochemical and biological properties feature structural diversity, which have led to a number of traditional and industrial applications. In recent years the interest in saponins has increased significantly because of their diverse properties as natural detergents and foaming agents, their cardiac, immunostimulating, and anti-cancer activity, as well as other health promoting functions<sup>1</sup>. Saponins are found in many plants, such as certain beans. Saponins are much more toxic to some creatures, such as fish, and hunting tribes have traditionally put large quantities of them in streams, lakes etc in order to stupefy or kill the fish<sup>2</sup>. Saponins comprise a large family of structurally diverse compounds containing a steroidal or triterpenoid aglycone linked to one or more oligosaccharide moieties. The aglycone or non-saccharide portion of the saponin molecule is called the genin or sapogenin. Depending on the type of genin present, the saponins can be divided into three major classes<sup>3</sup>.

a. Triterpene glycosides [triterpenoid saponozites (C<sub>30</sub>)] b. Steroid glycosides [steroidal saponozites (C<sub>27</sub>)]

- Spirostanol saponin
- Furostanol saponin
- Nugatigenin saponin
- Polipodo saponin

c. Steroid alkaloid glycosides (glikoalkoloids)

The chemically active ingredients in some plants appear to have a steroid like effect, meaning that they indirectly increase activity of steroidal hormones like testosterone and progesterone. The medicinal plants including *Dioscorea deltoidea*, *Bacopa monierri*, *Asparagus racemosus*, *Withania somnifera*, *Gentiana lutea*, *Smilax sarsaparilla*, *Glycyrrhiza glabra*, *Tribulus terrestris*, *Allium cepa* and *Capsicum annum* are used as potent precursors in the traditional system of medicine for infertility, low libido and impotence in both men and women. The biological activity elicited by these herbs are due to furostanol saponin<sup>4,5</sup>. The present research aims at determining and comparing the furostanol saponin content of each of the medicinal herbs.

## MATERIALS AND METHOD

### Collection of plants

Individual plants were collected and purchased from different regions of Kathmandu. The plants were authenticated by a qualified botanist in National Botanical and Herbarium Centre, Kathmandu. The specimens were also preserved with standard reference number. The specific parts of the plants were powdered individually using stainless steel domestic grinder to get powders (> 95% passed through 60#). These powders were used for extraction.

### Extraction of plant Materials<sup>6</sup>

Representative samples of plant material were powdered and ground materials (2 gm) extracted with chloroform (30 mL) with mixing, shaking and stirring for 1 hr. The extraction process was conducted in triplicate. The chloroform extracts were combined and solvent recovered under reduced pressure. Extracted plant materials were dried and powdered materials 1 gm extracted with 50 mL methanol for 1 hr using soxhlet apparatus. Using the same conditions, four successive extractions were performed. The extracts were pooled, filtered and concentrated under reduced pressure. Residue obtained dissolved in methanol and final volume adjusted to 100 ml with methanol.

### Reagent<sup>6</sup>

#### *P*- Dimethyl Amino Benzaldehyde Reagent

*P*- dimethyl amine benzaldehyde (1gm) was dissolved in 100 mL mixture of 4 N hydrochloric acid (34mL) and methanol (66mL).

### Incubation of extracts<sup>6</sup>

Representative extracts 5 ml each and reagent 5 mL were mixed in a stoppered glass tubes. Control sample containing 5 mL methanol and 5 mL reagent was prepared. Test and control samples were heated on water bath at  $58 \pm 2$  °C for 2 hr. After reaction the solutions were allowed to cool and absorption measured at 513 nm against the control solution. Furastanol saponins % was calculated by the formula.

$$X = \frac{a_1 \times 0.2 \times k \times 100}{a_2 \times b \times v} \times 100$$

Where,

$a_1$  = Absorption of the sample

$a_2$  = Absorption of the control sample

k = Coefficient 0.00555

b= Quantity of raw material

v = Volume analyzed

## RESULTS

By using a chemical assay procedure, the active saponin fraction (furostanol) in the herbs was quantified. The results showed that high furastanol saponin contents 5.26% was found in the *W. somifera* followed by *B. monierri* which is 5.11%. Similarly the contents of furastanol saponin recorded in *T. terrestris* (4.93%), *D. deltoid* (4.54%) and *S. sarsaparilla* (4.01%). A relatively low concentration of the furastanol concentration was found in *G. glabra* (3.67%), *G. lutea* (3.12%), *A. racemosus* (3.09%), *A. cepa* (3.02%) and *C. annum* (2.66%).

**Table: Furastanol Saponins contents in the Selected Herbs**

SN	Plant species	Furastanol Saponins %
1.	<i>Dioscorea deltoida</i>	4.54
2.	<i>Bacopa monierri</i>	5.11
3.	<i>Asparagus racemosus</i>	3.09
4.	<i>Withania somnifera</i>	5.26
5.	<i>Gentiana lutea</i>	3.12
6.	<i>Smilax sarsaparilla</i>	4.01
7.	<i>Glycrrrhiza glabra</i>	3.67
8.	<i>Tribulus terrestris</i>	4.93
9.	<i>Allium cepa</i>	3.02
10.	<i>Capsicum annum</i>	2.66

## DISCUSSION

Although the raw material used in the manufacture of products used for the treatment of infertility, low libido and importance are available in the country, yet these are not fully utilized for preparation of standard preparations. The use of synthetic preparations like Sildenafil Citrate has been thought to be effective treatment for male sexual dysfunction. However one should consider that Sildenafil Citrate carries risks and can create potentially dangerous side effects. Other considerations are, Sildenafil is not recommended for female sexual dysfunction and for many, cost prohibitive. In this regard relying on natural remedies would be beneficial for future prospects. Effective and extensive protocols should also be developed for extraction, purification and bioassay of natural products.

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