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ISOLATION, PURIFICATION AND PHARMACOLOGICAL STUDIES OF SAPONINS FROM A MEDICINAL PLANT *BRYONOPSIS LACINIOSA*

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Bryonopsis laciniosa commonly known as Shivalingi belongs to family cucurbitaceae and is an annual herb found throughout in India. Ethanobotanical litrature of India suggests the use of plant for treatment of inflammations and also to regenerate germinal epithelium in both males and females, producing their reproductive organs respectively. The whole plant is of pharmacological importance and is considered to have toxic properties .In the present studies extraction ,isolation ,purification and pharmacological effects were seen .Extration was done by defattining the whole dried plant by petroleum ether and then subjecting it to methanol extraction in soxhlet extractor for 18-20 hrs. Isolation of saponins was done with partition fractionation twice, first by using solvents chloroform and carbon tetrachloride and then with n-Butanol and water. n-Butanol fraction contains saponins as confirmed by TLC .Purification was done by affinity polarity by increasing ratio of $CHCl_3$: CH_3OH : H_2O from 65 : 20 : 10 to 65 : 35 : 10, respectivetly .Elution was done by using 80 % methanol . Presence of saponins was checked by TLC. Like fractions were pooled together and were concentrated. The concentrate was then further purified by coloumn chromatography and was designated as compound 1 . This compound 1 was used for pharmacological studies, such as antibacterial, antifungal, anti-inflammatory, diuretic and sperm immobilization effects.

Keywords : *Bryonopsis laciniosa;* Coloumn chromatography; Compound 1 and pharmacological effects; Fraction; Purification; Saponins; TLC.

Introduction

Saponins belong to a class of natural products and are marked by a number of common properties. Saponins are structurely constructed of aglycons (triterpenes or steroids) and glycones (sugars). Monodesmosidic saponins have single sugar chain normally attached to C-3 of triterpene or steroid, whereas didesmosidic saponins have two sugar chains attached to C-3 and C-28. Tridesmosidic saponins have three sugar chains and are rarely found in plants. Saponins are biological detergents because of glycosylation of hydrophobic aglycone and when agitated in water forms soapy lather that gives the name of the group of compounds.Their other properties are haemolytic activity ,toxicity to fish,complex formation with cholesterol and the newely found antibiotic properties.

1730 species belonging to 104 families where found to have 627 triterpenes and 127 steroidal saponins. Saponins play an active role in reduction of alcoholism[1]. Yang Xiuwei *et al.*[2] reported inhibition of the activity of HIV-1 protease by bioactive triterpenoid saponins of seeds of *Aesculus chinensis*. Sang *et al.*[3] isolated saponins from *Allium tuberosum*. Gabiorowski *et al.*[4] extracted the saponins from roots of *Silene vulgaris*, both saponins exhibited immunosuppressive anti-inflammatory and cytotoxic effects. Effect on zoospore motility was reported by saponins from *Panax notoginseng* and *Alphanomyces cochlioides*, were isolated by Wei *et al.*[5]. Saponins from *Acacia victoriae* induced apoptosis by mitochondrial perturbation.[6]. Yoshikawa *et. al.*[7] isolated a saponin from *Gymnema alternifolium* and determined its structure spectroscopically and by chemical evidence .This saponin had antisweet activity . Quantitation of saponins in arial subterranean tissues of *Medicago truncatula* was done by David *et al.*[8]. Zang *et al.*[9] extracted and isolated eight saponins from *Aesculus chinensis* and determined their structure by spectral data.

Techniques like HPLC, ID- NMR, 2D- NMR and FAB-MS spectrometry have been used by many workers[10] for characterization of saponins from many plants.

Medicinal properties of these saponins are described in "The Dictionary of Chinese Crude Drugs"[11]. Saponins from the bark of *Daniella oliveri* were found to be effective in cardiac problems[12]. NMR studies of *Randia*

Table 1. Results of polarity specific purification of crude saponin of *Bryonopsis laciniosa* by column chromatography.

S.No.	Fraction No.	Solvent System			Compound 1
		$CHCl_3$	CH_3OH	H_2O	
1	1-4	65	20	10	blank
2	5-9	65	20	10	Impurities
3	10-16	65	20	10	Compound 1 + fractional impurities
4	17-22	65	35	10	Compound 1 + Compound 11
5	23-28	65	35	10	Compound 1 + fractional molecules
6	29-36	65	35	10	Compound 111 + very little impurities
7	37-42	65	35	10	Compound 111+ Free sugar
8	43-48	80 % Methanol			Free sugar

siamensis showed pharmacological effect on decreasing blood pressure and increasing heart rate and contraction of uterus[13]. GC-MS SPME profiling of rhizobial bacterial volatiles reveal prospective induction of growth promotion and induced systemic resistance in plants as reported by Farang *et al.*[14]. Many other saponins from plants showed anti- fungal, anti -bacterial, anti-diuretic, anti-pyretic effects and decline in sperm motility.

Materials and Methods

Plant Materials -The whole fresh plant of *Bryonopsis laciniosa* (4 kg) was collected from R.D.V.V and T.F.R.I. campus, Jabalpur during summers. The plant material was shade dried, finelly chopped ,powdered and sieved as per standard procedure.

Extration and Isolation -Plant powder was defatted with petroleum ether at 40-60 °C for 18-20 hrs and then extracted with methnol at 40-50° C in Soxhlet extractor for 12-15 hrs. Concentrated extract was fractionated twice, first with $CHCl_3$ and CCl_4 to remove impurities, and then with n-Butanol and water. n- Butanol layer was designated as Fraction 1 which contains saponins. Aqueous layer was designated as Fraction 11 and it contained free sugars.

Study of Fraction 1 - For the butanol fraction i.e the fraction 1, the solvent system ethyl acetate : methanol : water (65:40:10) was used to examin it by TLC using 20% aqueous H_2SO_4 spray .Three pink spots appeared on spraying and were identified as saponins by their respective Rf values.

For separation of individual saponins, crude saponin mixture (fraction 1) was *vacuo* evaporated to dryness and was redissolved in methanol which was poured dropwise into large volume of ether – acetone (1:2) with constant stirring .The precipitate was collected by filtration and was coloum chromatographed on silica gel (100gms) using solvent system $CHCl_3$: CH_3OH : H_2O (65: 20: 10) increasing polarity upto (65: 35: 10) and finally the elution was done by 80% methanol .A total of 48 fractions of 20ml volume were collected.Saponins were pooled together and were concentrated. This was designated as compound 1.

The coloumn employed for purification had:

Length of coloumn 60 cm
Diameter of coloumn 1.5 cm
Weight of silica gel 100 gms
Weight of crude saponin 3 gms
Solvent system $CHCl_3$: CH_3OH: H_2O
Volume of each fraction 20 ml

(Results shown in Table 1)

Study of Compound - Isolation and purification of saponin yielded a white amorphous powder showing M.P.277° C and Rf value 0.28.

The compound was further confirmed to be triterpene by following tests :

1. Saponin specific test: On shaking compound 1 in aqueous medium the compound gave foam which lasted for some time . This confirmed it as saponin.

2. Feigal test for triterpene: A positive test was obtained by Feigal test. This confirmed it as triterpene.

Pharmacological Tests of Sponins

1. Anti-bacterial activity : Test bacteria *Staphylococcus aures* and *Escherichia coli* were maintained on nutrient agar slants (composition peptone 5.0 gms / lit ,beef extract 3.0 gms , agar 20 gms, pH 7.2, distilled water 1000 ml) Testing was done in nutrient broth (composition as above minus agar). After inoculation of loopful of culture from slants the broth were inoculated at 37 ±10° C for 24

hrs .The test saponin was dissolved in distilled water to obtain a 10 mg / ml extract solution. 0.2 ml solution of test material was added to 1.8 ml of the seeded broth and this forms the first dilution. 1ml of this was further diluted with 1ml of seeded broth to produce the second dilution and so on , till six such dilutions were obtained. A set of tubes containing only seeded broth was kept as control and suitable solvent controls were also maintained . Results were checked after 24 hrs of incubation. It was found that saponin concentration of 0.55 mg / ml or less had no growth inhibition effect on *Staphylococcus aureus* .bacteria.

2. Anti- fungal activity : Media for fungal growth was prepared by taking extract of 250gms of peeled, cut and boiled potato pieces in distilled water (500ml). 20gms of dextrose was added to it and volume was made 1 litre with distilled water . This was steri lised and then pH was maintained at 7.0 .In this media 6-7 days old fungal culture of *Aspergillus* ,*Alternaria* and *Colletotriculum* were inoculated and incubated in cooling incubator (Remi) at 27 ± 10^{0}C .Different concentrations of saponin were prepared (0.125ml, 0.25ml, 0.50ml and 1ml).1ml each was added to above said fungal growths in triplicates Observations were taken after 18 days .

3. Spermicidal activity : Spot test was employed for testing spermicidal activity.1% solution of saponin was prepared in the Sorensens phosphate buffer (pH 7.0-). One drop of human semen was placed on a slide. To this was added two drops of saponin solution, and was mixed thoroughly with a glass rod and a cover slip was placed over it. When seen under microscope the sperm were found to be partially immobilized.

4. Anti-inflammatory activity : Carrageanin induced oedma test was employed.The test saponin was administered to the group of five rats. After one hour 0.025 W of 1.0% carrageanin solution was injected subcutaneously into one of the hind paws of mice. After three hours the rats were sacrificed with an oral dose of ether and the paws were cut identically at the ankle joint and weighed. The difference between the weight of the two paws gives the amount of odema developed .It was compared with the control group which received carrageanin only and the anti-inflammatory effect caused by the compound was calculated . No significant anti-inflammatory activity was recorded.

Results and Discussion

Isolation of saponin is fairly standarised procedure and a score of workers have utilized it[15]. Some reviews outline the procedure[16] . Before isolation of saponin it is essential to remove fatty acids and lipid with petroleum ether, and then from defatted powder saponin was extracted with methanol .This step involves extraction of two types of molecules , one is saponin and the other is free sugars ,which were further separated by partitioning with n- Butanol and water . Saponin comes in n-Butanol and sugar in aqueous fraction . Both fractions were concentrated and studied further.

Methanol along with saponin, extracts other molecules like triterpenes which are further purified by polarity specific coloumn chromatography, by increasing the polarity of the solvents. This enables the desired saponin to be separated from other undesired triterpenes. Presence of saponin was checked by TLC. Elution was done with 80% methanol. This purified methanol extracted saponin fraction is different from other purified extract and is quiet specific for oleanic acid type of ring structures[17].At least some workers tend to attribute such structure to this molecule right away following the above described isolation , purification steps[18].

The whole saponin molecule was obtained by recrystallisation of isolation products in powder form having melting point 277^{0} C. The saponification process of the compound gave a clear insight of its being a saponin. Also a positive and unambiguous Fiegal test confirmed it as a saponin. Further a Lieberman-Burchard test gave a clear clue to the compound being a saponin. The anti microbial studies of plant *Bryonopsis laciniosa* showed that it had an effective bioactive molecule .A saponin concentration of 0.55 mg /ml or more could effectively stop the growth of *E. coli* and a concentration of 0.41 mg /ml or more stopped the growth of *S. aureus* bacteria. However, a relatively high concentration of saponin had anti-fungal effect .The spermicidal activity of this saponin was seen partially. No dieuretic and anti –inflammatory actively was seen.

Pharmacological properties observed and given here can be explained by the structure given 3-O [β-D-galactopyranosal-(1-2) glucopyranosyl].The molecular weight of this saponin was found to be 785[10].

Saponins are glycocidic compounds which have a very wide distribution in plants and constitute one of the most frequently occurring group of secondary metabolites. They are readily identified by their surface active properties and by their haemolytic characterstics and have been widely used since times imemmorable as pharmacological agents of wide spread uses . Saponins are attributed with such critical medicinal properties as lowering of cholesterol concentrations. Among the key biochemical effects of this saponin are increase in serum protein synthesis[19] and it stimulates RNA[20] and

cholesterol synthesis[21].

Various saponins from many plants showed anti-fungal anti-bacterial, anti–inflammatory, anti-pyretic and diuretic effects. Some saponins from plants were effective in cardiac problems and inhibition of HIV-1 protease. Saponins occur in numerous varities of plants and it is widely believed by phytochemists that the varieties and classes of saponins could be much more then reported. Henceforth, this gives rise to an interesting possibility of many more structures of saponins to be uncovered and numerous other pharmacological uses to be discovered.

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