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Evaluation of Antigonorrhoeal Activity of Saponins Extract of *Sapindus Mukorossi* Gaertn

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ABSTRACT

Sapindus mukorossi Gaertn. (Sapindaceae) locally known as “Reetha” has the reputation of being used as a medicinal herb against sexually transmitted diseases in Nepal. Using the mean zone of inhibition by agar well diffusion method and minimum inhibitory concentration (MIC) by test tube dilution method the powdered leaf extract was tested against *Neisseria gonorrhoeae*. The ethanolic and methanolic extract of the leaves of this plant was found to have antigonorrhoeal activity. The maximum mean zone of inhibition for ethanolic and methanolic extract was 12.5 ± 0.5 mm and 9.5 ± 0.4 mm respectively. The active ingredient of the ethanolic extract of the plant was very effectively extracted. A combination of frothing test and thin layer chromatography showed that saponins were present in the ethanolic elutes. The purified saponin extract of *S. mukorossi* Gaertn. were screened for antigonorrhoeal activity. The saponin extract inhibited the growth of *N. gonorrhoeae* in concentrations of $1000 \mu\text{g/ml}$. It was concluded that this characteristic of saponin has opened the avenues for the discovery of new clinically effective antigonorrhoeal compound.

Key words: *Sapindus mukorossi*, saponins, antigonorrhoeal activity, *Neisseria gonorrhoeae*.

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INTRODUCTION

Sapindus mukorossi Gaertn. a member of the family Sapindaceae commonly known as 'reetha' found in temperate to tropical regions throughout North India and Nepal in hills and plains in deep clayey loam soil with areas experiencing nearly 150 to 200 cm of annual rainfall. It is a deciduous tree widely grown in upper reaches of Indo-Gangetic plains, Shivaliks and sub Himalayan tracts at altitudes from 200 to 1500m.

Sapindus mukorossi Gaertn. is known for its antimicrobial properties that are beneficial for septic systems. In folk remedies this herb has the reputation for using as a mucolytic agent, emetic, contraceptive, for the treatment of excessive salivation and to treat chlorosis. The literature reveals this herb is used for the treatment of eczema, psoriasis, for removing freckles and for the sexually transmitted diseases [1]. In our preliminary screening study it has also been seen that the ethanolic extract of *S. mukorossi* successfully inhibit the growth of the clinical isolates of *N. gonorrhoeae* [2].

Saponins are glycosides occurring mainly in plants. They are abundant in many foods consumed by animals and man [3]. Saponins are divided into two groups: steroidal saponins which occur as glycosides in certain pasture plants such as *Brachiaria decumbens* and *Panicum* sp., and triterpenoid saponins, which occur in soybeans and alfalfa [4]. Recent research has established saponins as the active components in many herbal medicines [5, 6]. Many pharmacological activities have been reported about saponins such as antibacterial antifungal, antiviral, hepatoprotective anti-inflammatory and anti-ulcer [7 – 15].

Gonorrhoea is one of the classical sexually transmitted disease (STD) with human as the host for the causative agent, *N. gonorrhoeae*. According to Family Health Division, Ministry of Health, Nepal, gonorrhoea is one of the prevalent STD in Nepal [16]. The problem is further compounded by the emergence of resistance to antimicrobial agents that are commonly used against *N. gonorrhoeae*, making the treatment expensive and prolonged [17]. The development of resistant microorganisms on prolonged exposure to existing antimicrobial agents has been known for a long time [18]. This situation forced scientists to search for alternate antimicrobial substances, from plants which are cheap, readily available for the population, and have minimum side effects. The World Health Organization (WHO) also supports the use of medicinal plants provided it is proven to be efficacious and safe [19].

Nepal is rich in all the 3 levels of biodiversity, namely species diversity, genetic diversity and habitat diversity. Among the 7000 species of medicinal plants recognized all over the world, more than 900 types of precious medicinal plants are said to be found in Nepal [20]. Unfortunately, there is little or no scientific information concerning the antigonorrhoeal activity of *S. mukorossi* Gaertn. in Nepal.

Antimicrobial drug resistance in bacterial pathogens is of national and international concern. Therefore in the present paper an attempt has been made to determine the concentration of saponins in *S. mukorossi* Gaertn and its antigonorrhoeal activities.

MATERIALS AND METHODS

The leaves of *Sapindus mukorossi* was collected from Parsa (Birgunj) and Makwanpur (Hetauda) districts (altitude about 1500ft from above the sea level), and processed at Clinical Microbiology laboratory of National Medical College and Teaching Hospital, Birgunj, Nepal. These plants were identified on the basis of ethnobotanical knowledge of the particular area, available literatures [21, 22] and in the Department of Botany, Vidyasagar University, India.

Preparation of Plant Extracts

The active ingredient of the plant used in this study was very effectively extracted by using water, ethanol and methanol. Hence in this study the organic solvent (ethanol and methanol) was used as the extracting solvent. The leaves of the plant *S. mukorossi* Gaertn. were washed with distilled water, air dried at room temperature and grounded to fine powder by using grinder. The powdered material was stored in an air tight container until further use.

The solvent extracts were prepared by transferring 10g of the powder to sterile wide-mouthed screw-capped bottles containing the solvent. It was allowed to soak for 24 hours at room temperature then heated for an hour at 100°C. The mixture was then centrifuged at 2000 rpm for 10 minutes at 4°C. The supernatants were filtered through a sterile funnel containing sterile Whatmann filter paper no.1. It was filter sterilized using syringe filter containing 0.2µ cellulose acetate membrane (Sartorius) [23]. The percentage yield of the extract was determined using the expression:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of ground plant Material.}} \times 100 \quad [24].$$

Extraction and Isolation of compounds from the leaves of *S. mukorossi* Gaertn.

The powdered sample was defatted by using petroleum ether 3x1h at 40 °C. After filtering the petroleum ether, the sample was extracted with methanol 3x1h with mild heating. The combined methanol extract was concentrated and the methanol extract was dissolved in methanol – acetone mixture (1:5 v/v) to precipitate the saponins. The precipitate was dried under vacuum, turning to a whitish amorphous powder named as Crude Saponin Extract (CSE). The CSE was loaded on silica gel - 60 (230-400 mesh, Merck) chromatography column and eluted with chloroform-methanol-water (70:30:10). The first fraction collected was evaporated under reduced temperature; the resultant residue was called Pure Saponin Fraction (PSF). The presence of saponins in these fractions was detected by the characteristic frothing tests and thin layer chromatography.

Isolation of *Neisseria gonorrhoeae*

N. gonorrhoeae was isolated from male patients with acute gonococcal urethritis attending the Skin and Venereal disease department of National Medical College and Teaching Hospital, Birgunj, Nepal, which were direct smear-positive cases. For isolation of *N. gonorrhoeae*, urethral swabs were inoculated onto Modified Thayer Martin agar media (Hi Media, India) with vancomycin, colistin, nystatin and trimethoprim (VCNT) supplement (Hi Media, India). The inoculated culture plates were incubated at 36°C - 37°C in a moist atmosphere containing 5% CO₂ (candle extinction jar) for 24 – 48 hours. These consecutive clinical isolates were identified on the basis of colony morphology, gram staining, oxidase, superoxol and rapid carbohydrate utilization tests [25].

Preparation of the Inoculum

3 to 5 colonies grown on Modified Thayer Martin agar media were obtained. These colonies were inoculated in 2.5 ml Muller-Hinton broth in a test tube by rotating the straight wire at least ten times with the tip touching the bottom of the test tube. The turbidity is also matched with 0.5 McFarland Standard.

Assessment of *in vitro* antigonorrhoeal activity

The assessment of *in vitro* antigonorrhoeal activity of crude ethanolic extracts and the saponin fraction in this study was conducted at the Department of Microbiology, National Medical College and Teaching Hospital, Birgunj, under the supervision of Dr. Jagadish Narayan Shivapuri.

Antigonorrhoeal activity of *S. mukorossi* extracts

Muller Hinton chocolate agar plates with 5% sheep red blood cells were swabbed all over the surface with freshly prepared inoculum, using sterile cotton swab. Three wells (6 mm. diameter) were bored in the medium with the help of sterile cork-borer and were labeled properly. Fifty micro-liter (µl) of each solvent extracts of a medicinal plant was added in each well. Plates were left for 5 minutes till the extract diffuse in the medium and then incubated at 37°C in a moist atmosphere containing 5- 10% CO₂ for 48 hours. The antigonorrhoeal activity of the plant extracts were recorded by measuring the inhibition zones in millimeters with a measuring scale. The whole process was repeated in triplicate.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentrations (MICs) are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. MICs are used by diagnostic laboratories mainly to confirm resistance, but most often as a research tool to determine the *in vitro* activity of new antimicrobials and data from such studies have been used to determine MIC breakpoints [26].

The selected plant extracts were used for the determination of minimum inhibitory concentration (MIC) by the test tube dilution method. Appropriate dilutions of 45 – 1000 µg/ml were made to give a final volume of 1ml in the tubes. One drop equivalent to 0.02 ml of organisms (prepared as previously described) [27] was added to each test tube. A tube was set up without the extract as a control. The test tubes were incubated at 36°-37°C in a moist atmosphere containing 5% CO₂ in CO₂ incubator for 24 – 48 hours.

Determination of Minimum Inhibitory Concentration of Saponins

To detect the Minimum Inhibitory Concentration (MIC) of *S. mukorossi* Gaertn. saponins extract, seven sterile capped tubes of 7 x 1.3 cm were arranged in a row, with one row for the organism *N. gonorrhoeae*. 1000 mg *S. mukorossi* Gaertn. saponin extract was diluted in 10 ml of 0.1% peptone water to obtain a working solution of 100 mg/ml of saponins. Serial dilutions of the working solution were made. 1 drop of overnight broth culture of *N. gonorrhoeae* was delivered into each tube and incubated at 36°-37°C in a moist atmosphere containing 5% CO₂ in CO₂ incubator for 24 – 48 hours. The MIC was regarded as the lowest concentration that inhibited visible growth. Ciprofloxacin and Tetracycline were used as standards for the bacteria.

Thin Layer Chromatographic examination of Saponin

The saponins extracts were subjected to thin-layer chromatography (TLC) on silica gel plates (0.25 mm silica gel) using the solvent system methanol/distilled water (4:1). The developed plates were dried at room temperature. Visualization of saponin on developed plates was done by spraying with 50% (v/v) sulphuric acid. The sprayed chromatograph were allowed to dry for 15 min at room temperature and then heated at 105°C for 3 min in oven until the colour developed reached its maximum.

Frothing test was done by diluting the extract with 20ml. distilled water and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Statistical analysis

To ensure consistency of all findings, antigonorrhoeal activity of ethanolic, methanolic and saponin extract was performed in triplicate under aseptic condition. Data were analyzed and expressed as mean ± standard deviation.

RESULTS

S. mukorossi Gaertn. used in this study is widely used in folkloric medicine of Nepal in treating different diseases [21, 22]. In this study ethanolic, methanolic and aqueous extracts from the leaves of the plant was studied. The colour, consistency and yield of different extracts were given in table 1. The antigonorrhoeal activity of the plant extracts were recorded by

measuring the growth of inhibition zones in millimeters with a transparent ruler. The whole process was repeated in triplicate for determining the mean zone of inhibition. Among the different solvent extracts of the plant *S. mukorossi* ethanolic extract was found to be more sensitive against *N. gonorrhoeae*, followed by methanolic and aqueous extract. The maximum mean zone of inhibition was seen for ethanolic extract (12.5 ± 0.5 mm), followed by methanolic extract (table 3 and 5). The aqueous extract did not show any activity against the test organism *N. gonorrhoeae*.

Table 1: The colour, consistency and yield of different extracts of *S. mukorossi* Gaertn.

Sl. No.	Different Solvents	Colour of Extracts	Consistency	Yield (%age) Extractive Value*
1.	Ethanolic	Brown	Sticky	21.56 ± 0.67
2.	Methanolic	Brown	Sticky	16.57 ± 0.83
3.	Aqueous	Greenish	Dry	25.81 ± 0.56

*Values are in terms of Mean \pm SEM of results done in triplicate

From Table 2 it is concluded that among the five clinically isolated strains of *N. gonorrhoeae* two were sensitive at very lower doses against the ethanolic extract of *S. mukorossi*. The other three strains were sensitive at higher doses. Whereas among the two WHO isolate of *N. gonorrhoeae*, both of the isolates were sensitive against the ethanolic extract of *S. mukorossi* at the concentration of 500 μ g/ml.

Similarly from Table 4 it is concluded that among the five clinically isolated strains of *N. gonorrhoeae* only one was sensitive at very lower doses against the methanolic extract of *S. mukorossi*. The other two strains were sensitive at higher doses. Rest of the two strains did not showed any activity within these range. Whereas among the two WHO isolate of *N. gonorrhoeae*, both were sensitive against the methanolic extract of *S. mukorossi* at the concentration of 500 μ g/ml.

The MIC of ethanolic and methanolic extract of the leaves of *S. mukorossi* Gaertn. for different strains of *N. gonorrhoeae* is measured taking Ciprofloxacin and Tetracycline as standard (table 3 and 5). The lowest MIC of the ethanolic extract are 300 μ g/ml and 500 μ g/ml against *N. gonorrhoeae* DBN 1, DBN 2, DBN4 and DBN 5 strains respectively (table 3). The lowest MIC of the methanolic extract is 300 μ g/ml and 500 μ g/ml against *N. gonorrhoeae* DBN1, DBN2 and DBN5 strains respectively (table 5). Whereas *N. gonorrhoeae* DBN 3 and DBN 4 strains did not show any activity within these range.

The minimum inhibitory concentration of ethanolic and methanolic extract of the leaves of *S. mukorossi* Gaertn. reveals that all the strains of *N. gonorrhoeae* are sensitive to the ethanolic extract, only three strains are sensitive to methanolic extract. On the basis of this result only the active ingredient of the ethanolic extract of the plant was very effectively extracted. A combination of frothing test and thin layer chromatography showed that saponins were present in the 100% ethanolic elutes only. Thin-layer chromatography reveals one spot attributable to saponin. Stable foams were observed in the frothing test indicating the presence

of saponins. The saponin extract inhibited the growth of *N. gonorrhoeae* in concentration of 1000 and 1500µg/ml. The MIC of ciprofloxacin and tetracycline were used as standards for the bacteria for all the tests.

Table 3: Determination of MIC (Minimum inhibitory concentration) of the ethanolic crude extract of *S. mukorossi* Gaertn. against different strains of *N. gonorrhoeae*

Strains of <i>N. gonorrhoeae</i>	MIC of ethanol extract (µg/ml)	Zone of inhibition at MIC (mm)	MIC of Ciprofloxacin (µg/ml)	Zone of inhibition at MIC (mm)	MIC of Tetracycline (µg/ml)	Zone of inhibition at MIC (mm)
<i>N. gonorrhoeae</i> DBN 1	300	11.5±0.5	50	13	100	13
<i>N. gonorrhoeae</i> DBN 2	500	11.3±0.3	100	12	–	–
<i>N. gonorrhoeae</i> DBN 3	1000	11.5±0.5	150	12	–	–
<i>N. gonorrhoeae</i> DBN 4	500	10.4±0.4	–	–	–	–
<i>N. gonorrhoeae</i> DBN 5	300	12.5±0.5	50	10	100	12
<i>N. gonorrhoeae</i> WHO isolate SDJ 1**	500	11.3±0.6	50	13	100	13.5
<i>N. gonorrhoeae</i> WHO isolate SDJ 2***	500	11.0±0.5	150	11.5	–	–

N. gonorrhoeae DBN 1: Penicillinase producing *N. gonorrhoeae* (PPNG); *N. gonorrhoeae* DBN2 : Tetracycline resistant *N. gonorrhoeae* (TRNG) *N. gonorrhoeae* DBN3 : Penicillin and Tetracycline resistant *N. gonorrhoeae* (PPNG & TRNG); *N. gonorrhoeae* DBN4: Ciprofloxacin and Tetracycline resistant *N. gonorrhoeae* (CFNG, TRNG); *N. gonorrhoeae* DBN5 : Penicillinase producing *N. gonorrhoeae* (PPNG)

***N. gonorrhoeae* WHO isolate SDJ1: Penicillinase producing *N. gonorrhoeae*

****N. gonorrhoeae* WHO isolate SDJ2: Penicillinase producing and Tetracycline resistant *N. gonorrhoeae*

Table 4: Effect of Methanolic extract of *S. mukorossi* Gaertn. on different strains on *N. gonorrhoeae*.

Strains of <i>N. gonorrhoeae</i>	Growth in Muller Hinton Chocolate agar containing different concentration of methanolic extract of <i>S. mukorossi</i> (µg/ml)						
	0*	45	90	150	300	500	1000
<i>N. gonorrhoeae</i> DBN 1 ^s	+	+	+	+	–	–	–
<i>N. gonorrhoeae</i> DBN 2 ^s	+	+	+	+	+	–	–
<i>N. gonorrhoeae</i> DBN 3 ^s	+	+	+	+	+	+	+
<i>N. gonorrhoeae</i> DBN 4 ^s	+	+	+	+	+	+	+
<i>N. gonorrhoeae</i> DBN 5 ^s	+	+	+	+	+	–	–

<i>N. gonorrhoeae</i> WHO isolate SDJ 1**	+	+	+	+	+	-	-
<i>N. gonorrhoeae</i> WHO isolate SDJ 2***	+	+	+	+	+	-	-

0* : Control plate without drug; '+' : Growth; '-' : No growth

N. gonorrhoeae DBN 1: Penicillinase producing *N. gonorrhoeae* (PPNG); *N. gonorrhoeae* DBN2 : Tetracycline resistant *N. gonorrhoeae* (TRNG) *N. gonorrhoeae* DBN3 : Penicillin and Tetracycline resistant *N. gonorrhoeae* (PPNG & TRNG); *N. gonorrhoeae* DBN4: Ciprofloxacin and Tetracycline resistant *N. gonorrhoeae* (CFNG, TRNG); *N. gonorrhoeae* DBN5 : Penicillinase producing *N. gonorrhoeae* (PPNG)

***N. gonorrhoeae* WHO isolate SDJ1: Penicillinase producing *N. gonorrhoeae*

****N. gonorrhoeae* WHO isolate SDJ2: Penicillinase producing and Tetracycline resistant *N. gonorrhoeae*

Table 5: Determination of MIC (Minimum inhibitory concentration) of the methanolic crude extract of *S. mukorossi* Gaertn. against different strains of *N. gonorrhoeae*

Strains of <i>N. gonorrhoeae</i>	MIC of methanol extract (µg/ml)	Zone of inhibition at MIC (mm)	MIC of Ciprofloxacin (µg/ml)	Zone of inhibition at MIC (mm)	MIC of Tetracycline (µg/ml)	Zone of inhibition at MIC (mm)
<i>N. gonorrhoeae</i> DBN 1	300	8.8±0.3	50	8.5	100	8.0
<i>N. gonorrhoeae</i> DBN 2	500	7.5±0.5	100	8.0	-	-
<i>N. gonorrhoeae</i> DBN 3	-	-	-	-	-	-
<i>N. gonorrhoeae</i> DBN 4	-	-	-	-	-	-
<i>N. gonorrhoeae</i> DBN 5	300	8.8±0.3	50	7.5	100	8.0
<i>N. gonorrhoeae</i> WHO isolate SDJ 1**	300	9.5±0.4	50	10	100	8.5
<i>N. gonorrhoeae</i> WHO isolate SDJ 2***	>500	8.0±0.5	100	8.0	-	-

N. gonorrhoeae DBN 1: Penicillinase producing *N. gonorrhoeae* (PPNG); *N. gonorrhoeae* DBN2 : Tetracycline resistant *N. gonorrhoeae* (TRNG) *N. gonorrhoeae* DBN3 : Penicillin and Tetracycline resistant *N. gonorrhoeae* (PPNG & TRNG); *N. gonorrhoeae* DBN4: Ciprofloxacin and Tetracycline resistant *N. gonorrhoeae* (CFNG, TRNG); *N. gonorrhoeae* DBN5 : Penicillinase producing *N. gonorrhoeae* (PPNG)

***N. gonorrhoeae* WHO isolate SDJ1: Penicillinase producing *N. gonorrhoeae*

****N. gonorrhoeae* WHO isolate SDJ2: Penicillinase producing and Tetracycline resistant *N. gonorrhoeae* the value of MIC is > 500µg/ml.

Table 6: Antigonorrhoeal sensitivity test of *S. mukorossi* Gaertn. saponins.

Concentrations (µg/ml)	50	100	250	500	1000	1500	Control	Result
MIC of <i>S. mukorossi</i> saponins on <i>N. gonorrhoeae</i> WHO isolate SDJ 1**	+	+	+	+	-	-	+	Sensitive at 1000 µg/ml
MIC of <i>S. mukorossi</i> saponins on <i>N. gonorrhoeae</i> WHO isolate SDJ 2***	+	+	+	+	-	-	+	
MIC of Ciprofloxacin on <i>N. gonorrhoeae</i>	+	-	-	-	-	-	+	Sensitive at 100 µg/ml
MIC of tetracycline on <i>N. gonorrhoeae</i>	+	+	+	-	-	-	+	Sensitive at 500 µg/ml

***N. gonorrhoeae* WHO isolate SDJ1: Penicillinase producing *N. gonorrhoeae*

****N. gonorrhoeae* WHO isolate SDJ2: Penicillinase producing and Tetracycline resistant *N. gonorrhoeae*

DISCUSSION

The use of plants to heal diseases including infectious ones has been extensively applied by people. *S. mukorossi* Gaertn. belongs to the family Sapindaceae, which has not attracted many attention regarding sexually transmitted diseases. Data from literature as well as the present study results reveal great potential of plant extracts or fractions for the therapeutic treatment of gonorrhoea. Information on medicinal plants used for the treatment of gonorrhoea and reports on antigonorrhoeal activity of plants is very scarce [28]. The presence of antibacterial substances in the higher plants is well established [29]. Plant derived medicines have made a significant contribution towards human health. Earlier it has been seen that the susceptibility of gram negative bacteria to these plant derived medicines are very much limited compared to gram positive bacteria [30, 31].

Based on the mean zone of inhibition and MIC, different strains of the organism *N. gonorrhoeae* was found to be most susceptible to the ethanolic extract of *S. mukorossi* Gaertn. and least towards the methanolic extract. The results obtained indicate the existence of antimicrobial compounds in the crude ethanolic extract of *S. mukorossi* Gaertn. Water was used by the traditional healers as the solvent but we found in this study that the ethanolic and methanolic extract of *S. mukorossi* Gaertn. provided more consistent antigonorrhoeal activity compared to extract by water. This might be resulted due to the lack of solubility of the active constituents in aqueous solution. The low minimum inhibitory concentration observed for the ethanolic extract of *S. mukorossi* Gaertn. is of great significance in the health delivery system. It could also be used as an alternative treatment to orthodox antibiotics in the treatment of

gonorrhoeae, as the isolate *N. gonorrhoeae* frequently develop resistance to known antibiotics [17].

The result of the thin layer chromatography and the frothing test show that the ethanolic extract of *S. mukorossi* Gaertn. contains saponin in some significant amounts. The results also show that the saponin extract prepared from *S. mukorossi* Gaertn. possesses antigonorrhoeal property. The presence of the saponin in the ethanolic extract is an indicator that *S. mukorossi* Gaertn. can be a potential source of precursors in the development of synthetic drugs against gonorrhoeae. Likewise, it has been reported in 2005 that the extracts of plants are inhibitory to the clinical isolates of *N. gonorrhoeae* [32].

It is equally interesting to note that the saponins compared favourably with the standard antibiotics such as ciprofloxacin and tetracycline, which were used in this study. In addition, the saponin extract is a mixture of several saponins each of which could be as effective as or even more effective than the popular antibiotics currently being used after purification [4].

In recent years, antibiotic resistance against *N. gonorrhoeae* have increased dramatically and in our previous study we reported that out of the 30 isolates, 18 (60.0%) were resistant to penicillin, 10 (33%) were resistant of tetracycline and 6 (20%) were resistant to Ciprofloxacin [17]. Thus, the treatment of gonorrhoeae has become very difficult reducing the therapeutic options. Likewise, it is also very dangerous to use large dose of most synthetic drugs due to their toxicity while the body system can still accommodate some plant extracts at relatively high doses [33]. Therefore, bioactive substances from the plant under study can be employed in the formulation of antimicrobial agents for the treatment of gonorrhoea.

The result of the present study appears to have a scientific basis and justified the traditional use of the plant *S. mukorossi* Gaertn. against gonorrhoeae, amongst the people in the rural communities. Moreover low cost of the herbal preparation and easy accessibility of the studied plant ushers for the selection of the particular plant against gonorrhoeae.

CONCLUSION

The present study has demonstrated that saponin is present in *S. mukorossi* Gaertn. It also revealed that the saponin extract from *S. mukorossi* Gaertn. has useful antimicrobial properties. This finding is consistent with previous published reports that specific saponins could have antimicrobial properties [34, 35]. This characteristic of saponin has opened the avenues for the discovery of new clinically effective antigonorrhoeal compound. Saponins physicochemical and biochemical properties are increasingly being exploited in the pharmaceutical sector. With the availability of primary information, the future investigation should be directed towards the elucidation of the mechanism of action of the extract and the toxicological evaluation with the aim of formulating novel chemotherapeutic agents to cope up with increasing prevalence of drug resistant gonorrhoea.



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REFERENCES

- [1] Kirtikar KR, Basu BD. Indian Medicinal Plants. B.L.M. Publication, Allahabad, 1991.
- [2] Mondal KC, Bhargava D, Kar S, Shivapuri JN, Shakya B, Maity C. *Int J Pharma and Bio Sci*. 2011; 2/1.
- [3] Cheeke PR. *Canadian J Anim Sci* 1971; 51: 621-632.
- [4] Soetan KO, Oyekunle MA, Aiyelaagbe OO, Fafunso MA. *African J of Biotech* December 2006; 5: 2405-2407.
- [5] Liu J, Henkel T. *Curr Med Chem* 2002; 9: 1483–1485.
- [6] Alice CB, Vargas VMF, Silva GAAB, de Siqueira NCS, Schapoval EES, Gleye J, Henriques JAP, Henriques AT. *J Ethnopharm* 1991; 35: 165–171.
- [7] Oakenfull, Fenwick. *J Sc Food Agric* 1981; 32: 273-278.
- [8] Price, Fenwick. *Int Rev Food Sci Nutr* 1990; 157: 62.
- [9] Just MJ, Recsio MG, Gner RM, Cuellar MJ, Marez S, Bilia AR, Rios J. *Planta Med* 1998; 64: 404-407.
- [10] Chao AC, Nguyen JV, Broughall M, Recchia J, Kensil CR, Daddona PE, Fix JA. *J Pharm Sci* 1998; 87: 1395-1399.
- [11] Tschesche R, Wulff G. *Chem Org Naturist* 1973; 30: 461.
- [12] Jun HK, Park KY, Jo JB. *Chem Abstr* 1989; 106: 116-199.
- [13] Okubo K, Kudou S, Uchida T, Yoshiki Y, Yoshikoshi M, Tonomura M. *ACS Symp Ser* 1994; 546.
- [14] Arao T, Udayama M, Kinjo J, Nohara T. *Planta Med* 1998; 64: 413-416.
- [15] Zhang S, Hu Z. *Chem Abstr* 1985; 10: 512.
- [16] Ministry of Health, Government of Nepal. National Medical Standard For Reproductive Health Services. Family Health Division (August 2003).
- [17] Bhargava D, Shakya B, Mondal KC, Rijal BP. *J Inst Med* 2010; 32: 15 – 18.
- [18] Weisser R, Asscher AW, Winpenny J. *Nature* 1966; 219: 1365-1366.
- [19] World Health Organization (WHO). The World Health Report. Bridging the gap, WHO, Geneva, 1995, 1: pp.118.
- [20] Manandhar NP. *Plants and People of Nepal*. Timber Press, USA, 2000, pp 50.
- [21] HMG/N. *Medicinal Plants of Nepal*. Ministry of Forest and Soil Conservation, Department of Plant Resources, Kathmandu, Nepal, 1993.
- [22] Rajbhandari RK. *Ethnobotany of Nepal*. Ethnobotanical Society of Nepal, Kathmandu, 2001, pp. 98-134.
- [23] Krishnan K, Thenmozhi M, Vinitha Gunaseker. *Int J of Nat and Eng Sci* 2009; 3: 22-25.
- [24] Banzo A, Adeyemo S. *BIOKEMISTRI* 2006; 18: 39-44. Available online at <http://www.bioline.org.br/bk> and at <http://www.ajol.info/journals/biokem>. (June 2006).



- [25] Collee JG, Miles RS, Watt B. Mackie and McCartney Practical Medical Microbiology. Churchill Livingstone, London, 1996, pp. 140-141.
- [26] Andrews JM. J Antimicrobial Chemotherapy 2001; 48: 5 – 16.
- [27] Cruickshank R, Duguid JP, Marmion BP, Swain RHA. Medical Microbiology, Churchill Livingstone, Edinburgh, 1975, pp. 190.
- [28] Caceres A, Menendez H, Mendez E, Cohobon E, Samayoa EB, Jauregui E, Peralta E, Carrillo G. J Ethnopharm 1995; 48: 85-88.
- [29] Srinivasan D, Nathan S, Suresh T, Perumalsamy O. J Ethnopharmacol 2001; 74: 217-220.
- [30] Abu-Shanab B, Adwan G, Abu Safiya D, Jarrar N, Adwan K. Turk J Biol 2004; 28: 99-102.
- [31] Basri DF, Fan SH. Indian J Pharma 2005; 37: 26-29.
- [32] Shokeen P, Ray K, Bala M, Tandon VD, Ambedkar BR. Sex Transm Dis 2005; 32: 106 – 111.
- [33] Adebayo-Tayo BC, Adegoke AA. J Medicin Plants Res 2008; 2: 306-310.
- [34] Fenwick GR, Price KR, TsuKamoto C, Okubo K. Institute of Food Research, Norwich Laboratory. Publication NO 03249N, 1992, pp. 285- 326.
- [35] Campbell JB. Saponins: Adjuvants: Theory and Practical Applications. Butterworth-Heinemann Inc, Toronto, London, New York, 1993.