World Journal of Research and Review (WJRR)
ISSN:2455-3956, Volume-2, Issue-5, May 2016 Pages 89-93

Extraction of Saponins from Soapnut (*Sapindus Mukorossi*) and Their Antimicrobial Properties

Merve Deniz Köse, Oguz Bayraktar*

***Abstract*— In this study optimization of extraction conditions for saponin from *Sapindus mukorossi* was investigated. Results showed that polarity of the extraction solvent affects the yield percentage of the extraction process. Best yield percentage was obtained as 78.1 % at 1:10 solid-liquid ratio in aqueous ethanol solution (50% v/v). The antimicrobial properties of extracts containing saponins were investigated for different microorganisms. Minimum inhibition concentrations of extract were obtained against *Escherichia coli, Staphylococcus aureus* and *Candida albicans*. Minimum inhibition concentrations (MIC) of saponin extract ranged between 12.5 mg/mL to 25 mg/mL.**

***Index Terms*— Antimicrobial Properties, Saponin, Sapindus Mukorossi, Minimum Inhibition Concentration.**

I. INTRODUCTION

Saponins are commonly found in plants. Plant derived saponins are secondary metabolites mostly used in detergents due to its amphiphilic nature with the presence of a lipid-soluble aglycone and water-soluble chains in their structure [1]. Saponins divided into two groups which are triterpenoid and steroid glycosides. Glycosides structure characterizations are varied by the numbers of sugar units attached at different positions [2].

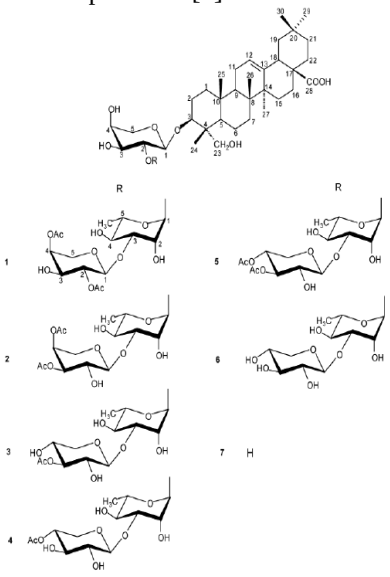

Figure 1 Saponins isolated from Sapindus Mukorossi [3]

Bioassay directed isolation and characterization of a new acetylated triterpene saponin, which named hederagenin 3-O-(2,4-O-diacetyl-α-L--arabinopyranoside)-(1-3)-α-L-rham nopyranosyl- (1-2)- α-L-arabinopyranoside (1), together with six known triterpenoidal hederagenin saponin (2-7) from *S. mukorossi* [3].

Ginseng is the most widely used saponin source in plant materials. Other plants containing saponin are; soymilk, sugar beet, chickpea, asparagus, soy, strawberry and plum fruit. Sapindus mukorossi derived saponins have inhibitory properties against golden apple snail, which are the major pests of rice and other aquatic crops in Asian countries [3]. Saponin works as a shield in the plant defense system to fight against pathogens and herbivores [4]. Consequently it is found in plant tissues that are most vulnerable to fungal or bacterial attack or insect predation [5].

Other than its traditional uses saponin has become one of the most interested subjects due their potential in industrial processes and pharmaceutical industry. The pharmaceutical purposes of saponin comes from their possession of antimicrobial properties [6], [7], their ability of increasing immune responses [8], [9], anticancer [10], antidiabetic and anti-obesity [11]. Other pharmaceutical properties of saponins are, hemolytic, molluscicidal, anti-inflammatory, antifungal or anti-yeast, antibacterial or antimicrobial, anti-parasitic, antitumor, and antiviral [12]. It works as a starting point for the semi-synthesis of steroidal drugs in pharmaceutical industry.

Apart from pharmaceutical applications, saponins have been used in food processes as a natural surfactant and serve as preservative in controlling microbial spoilage of food. More recently, due to natural substance preference, *Quillaja saponin* has been used as a natural small molecule surfactant in beverage emulsions in replacing synthetic surfactant of Tweens [13]. The effectiveness of the natural surfactant isolated from the bark of the *Quillaja saponaria* Molina tree for forming and stabilizing emulsions with a synthetic surfactant (Tween 80) has been compared [14]. Due to its foam-like characteristic saponins work as a natural bio-surfactant.

Increasing discoveries in pharmaceutical properties of saponin, especially anticancer, have intensified the seeking of saponins from plant materials. These have led to various new extraction technologies with the main purpose of maximizing the yield in order to procure the recent need.

In our study optimization of extraction conditions have been investigated. Saponin was extracted from *Sapindus Mukorossi*. And its antimicrobial properties have been studied and minimum inhibition concentration values were determined.

Merve Deniz Köse, Department of Chemical Engineering, Ege University, İzmir, Turkey
***Oguz Bayraktar**, Department of Chemical Engineering, Ege University, İzmir, Turkey
* Corresponding author

II. MATERIAL AND METHODS

A. Plant Materials and Chemicals

For Saponin source *Sapindus mukorossi* was used. In all extraction experiments analytical grade ethanol was used and purchased from Merck (Germany).For colorimetric assay vanillin was used and purchased from Fluka (USA). Analytical standard oleanolic acid was used and purchased from Fluka (USA). Sulfuric acid was purchased from Sigma-Aldrich. Nutrient broth and Nutrient agar were purchased from Fluka (USA) and used for bacterial reproduction.

B. Extraction of Plant Material

Sapindus mukorossi was dried at 80°C for three day. Dried *Sapindus mukorossi* was grinded into a powder. Extraction experiments were done at different solid-liquid ratios with different solvents. Solvents were used in extraction experiments, water at room temperature, boiling water, pure ethanol and ethanol water solution (50 % v:v). For extraction *Sapindus mukorossi* powder was added into the solutions with different solid-liquid ratios 1:10 and 1:20, respectively. For 1:10 solid-liquid ratio 10 g powder was added 100 mL of each solvent. For 1:20 solid-liquid ratio 5 g powder was added into 100 mL of each solvent. Solutions were mixed using magnetic stirrer for six hours.

C. Determination of efficiency of solvents

Powdered *Sapindus mukorossi* was extracted with different solvents at different solid-liquid ratios efficiency of the solvents were obtained. After extraction undissolved particles was removed from solution. Remaining solution was freeze dryed using a freeze dryer. After freeze drying obtained powder of *Sapindus mukorossi* extract was measured. And extraction yields of different solvents were calculated.

D.Determination of Saponin content of Sapindus mukorossi

Vanillin–Sulfuric acid assay was used to determine saponin content of *Sapindus mukorossi* extract. The procedure reported by Hiai et al. (1976) was used [15]. 0.5 mL of aqueous sample solution, 0.5 mL vanillin solution of 8% (w/v) and lastly 5.0 mL of sulfuric acid of 72% (w/v) were added and mixed in an ice water bath. After the mixture was then warmed in a bath at 60 ˚C for 10 minutes then cooled in ice–cold water. As a saponin standard oleanolic acid was used. Calibration curve was obtained for measured absorbance values at 527 nm.

D. High Performance Liquid Chromatography (HPLC) Analysis

Analysis of *Sapindus mukorossi* was done at reverse phase with mobile phase acetonitrile/water 40/60 to 60/40 in 60 minutes. The HPLC Equipment (Hewlett-Packard Series HP 1100) with a diode array detector was used. A C18 LiChrospher 100 analytical column (250 mm × 4 mm i.d.;with a particle size of 5 mm) thermo-stated at 30 ºC was chosen as stationary phase. The flow rate was 1ml min^{-1} and the absorbance changes were monitored at 280 nm.

E. Determination of Antimicrobial Activity and of Minimum Inhibition Concentrations (MIC)

Antimicrobial activities of *Sapindus mukorossi* for gram negative bacteria *Escherichia coli*, for gram positive bacteria *Staphylococcus aureus* were used. And *Candida albicans* was used as a fungus. The extracts were dissolved in sterile deionized water for a concentration of 50 mg/mL. Serial dilutions of extracts were done with a final concentration of 0.4 mg/mL. In each well of 96 well plates different extract concentrations and nutrient broth were added. Each well inoculated with bacterial subculture and incubated for six hours. In all antimicrobial experiments were carried out at negative and blank control conditions. Negative control was done only at nutrient broth and bacterial subculture suspension. Blank control was consisted only nutrient broth. Growth curves were obtained from turbidity of culture at 640 nm by UV spectrophotometer. MIC values of each extracts were determined by a standardized protocol of Varioskan microplate reader.

III. RESULTS AND DISCUSSION

A. Extraction Yields of Extraction Solvents

Extraction yield percentages were obtained for each solvent. Yield percentages were between 44-78 % for different solvents. In Table I yield percentages of solvents and solid-liquid ratios were tabulated. For 1:10 solid-liquid ratio the highest yield percentage was obtain in ethanol water solution. In 1:20 solid-liquid ratio for each solvent extraction yield percentage were clearly decreased. For better extraction yield 1:10 solid-liquid ratio was preferred.

Table I Yield Percentages of Extraction Solvents

Solvent Type	Solid- liquid ratio (g/ml)	Yield Percentage
Water	(1:10)	75,6
Water@100C	(1:10)	70,9
Ethanol-water	(1:10)	78,1
Ethanol	(1:10)	67,8
Aceone	(1:10)	65,0
Water	(1:20)	74,2
Water@100C	(1:20)	69,9
Ethanol-water	(1:20)	65,6
Ethanol	(1:20)	44,6

B. HPLC Analysis

For pure water and ethanol-water solution HPLC analysis were done at 280 nm. In Fig. 2 peaks of saponin in pure water as an extraction solvent were observed. As shown in Fig. 2 there were eight peaks observed from 0 to 60 min for *S.mukorossi* saponins. However four distinct peaks were chosen and area of the peaks were calculated. For 36, 41, 45 and 49 minutes area percentage of the peaks were calculated as 19.2, 13.7, 17.6 and 44.5 %, respectively.

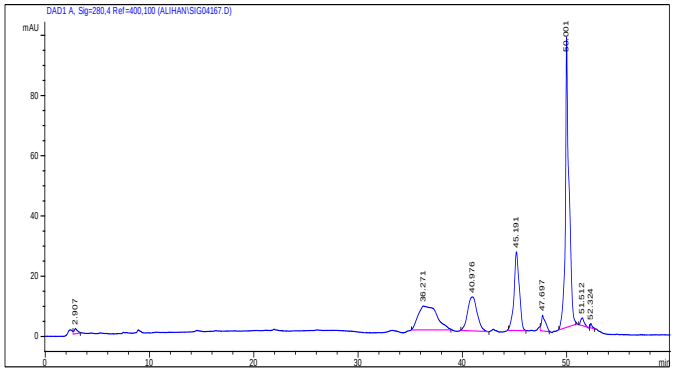

Figure 2 HPLC chromatograms of *sapindus mukorossi* extract in water.

In Fig. 3 peaks of saponin in ethanol water solution as an extraction solvent were observed. As seen from Figure 3 four distinct peaks were observed and area of the peaks were calculated. For 36, 41, 45 and 49 minutes area percentage of the peaks were calculated as 15.5, 10.5, 18.3 and 54.5 %, respectively. The number of peaks was close to seven and six hedragenin saponins were reported by Huang et al. (2003) [3] and Saxena et al. (2004) [16] for Indian *S. mukorossi*. Due to lack of standard of saponin content comparisons were done with literature. The peaks were obtained at 280 nm believed to be saponin peaks. Difference might be due to different HPLC method used in this study.

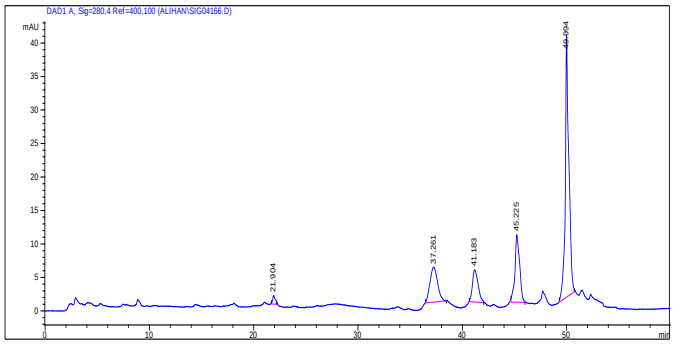

Figure 3 HPLC chromatograms of *sapindus mukorossi* extract in ethanol water solution.

Comparing both area of percentages at different solvents the result showed that by changing polarity of the extraction solvent, area of peaks for same material changed. Area of peaks observed at 36 and 41 minutes decreased when polarity of the solution was increased. Accordingly areas of peaks observed at 45 and 49 minutes increased when polarity of the solution were increased. These data show that polarity of extraction solvent affects the results of extraction significantly. Therefore it is crucial to choose proper solvent for optimization of the extraction process.

C. Determination of Saponin Concentration in Sapindus Mukorossi Extract

As explained in experimental method using colorimetric assay saponin concentration in *Sapindus Mukorossi* extracts. For calibration curve as a standard oleanolic acid was used. Measurements were done with spectrophotometer at 527 nm. Amount of saponins were given accordingly equivalent amount of oleanolic acid. In Table II for each solvent, extracted saponin amount were given. As seen in the Table II ethanol-water solution was the best extraction solvent for efficient extraction of saponin from *Sapindus Mukorossi.*

Table II Saponin Concentration in *Sapindus Mukorossi* Extract

Solvent Type	Solid- liquid ratio (g/ml)	g oleanolicacid/g extract	g saponin/g raw mat.
water	(1:10)	0,398	0,30
water@100C	(1:10)	0,368	0,26
ethanol-water	(1:10)	0,443	0,35
ethanol	(1:10)	0,361	0,24
aceone	(1:10)	0,401	0,26
water	(1:20)	0,356	0,26
water@100C	(1:20)	0,397	0,28
ethanol-water	(1:20)	0,416	0,27
ethanol	(1:20)	0,425	0,19

As given in the literature saponin amout in *Sapindus mukorossi* ranged between 10%-11.5% [17]. Due to solvent difference in our result saponin extract ranged between 19%-30%.

D. Minimum Inhibition Concentration (MIC) Values

In this study antimicrobial properties of *Sapindus Mukorossi* were examined for their minimum inhibition concentration (MIC) against gram negative bacteria, gram positive bacteria and fungus. To determine the MIC values serial dilution of extract was used in 96 well plates. Measurements were done with microplate reader (Thermo Varioskan). Minimum inhibition concentration data were obtained for each microorganism. MIC values were between 12.5 mg/mL to 25 mg/mL. From Fig. 4, 5 and 6 the highest growth curve was the negative control conditions which included only nutrient broth and microorganisms. The highest growth curve shows that maximum microorganisms activity in the well culture without any inhibitions. Minimum inhibition concentration is defined as the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism during incubation. [18].

As seen in Fig. 4 growth of *E.coli* was affected with the addition of extract to the well culture. With increasing the extract amount in the culture *E.coli* growth were decreased. Minimum inhibition concentration for *E.coli* was observed as 12.5 mg/mL.

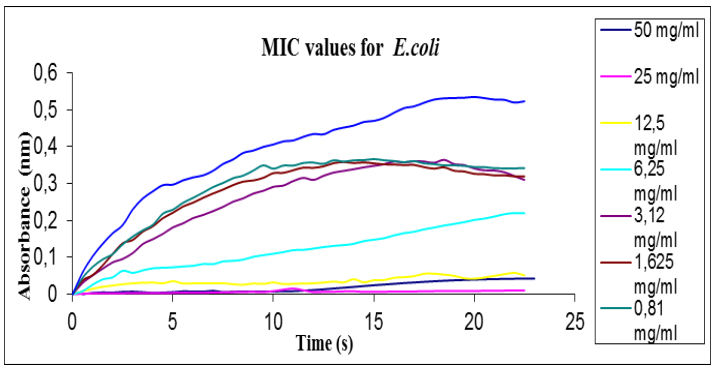

Figure 4 Minimum Inhibition Concentration values for *E.coli*

In Fig. 5 MIC values for *S.aureus* was given. Similar to the *E.coli*, growth of *S.aureus* was decreased with increasing amount of extract in the well culture. Slightly different from *E.coli* 12.5 mg/mL was not as effective on *S.aureus* as it was

on *E.coli*. Minimum inhibition concentration for *S.aureus* was observed higher concentration than 12.5 mg/mL. This difference might be occurred due to the fact *E.coli* is a gram negative bacteria but *S.aureus* is a gram positive bacteria.

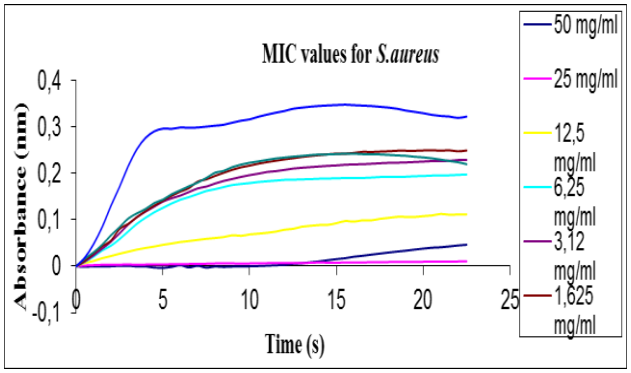

Figure 5 Minimum Inhibition Concentration values for *S.aureus*

In Figure 6 MIC values for *C.albicans* is given. Similar to the both bacteria growth of *C.albicans* was decreased with increasing amount of extract in the well culture. With increasing the extract amount in the culture *C.albicans* growth were decreased. Minimum inhibition concentration for *C.albicans* was observed as 12.5 mg/mL.

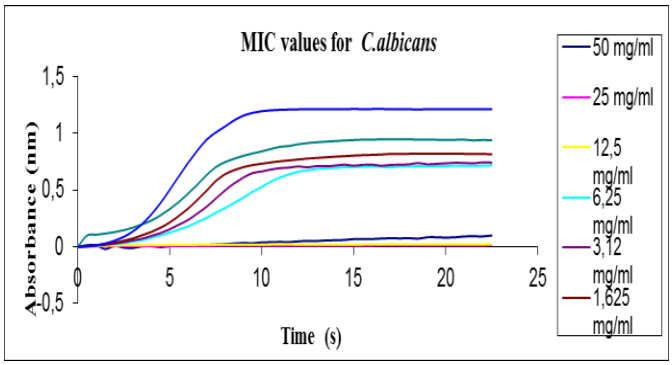

Figure 6 Minimum Inhibition Concentration values for *C.albicans*

As shown in our results antimicrobial properties of saponin were effective on different microorganisms.

By comparison with literature antimicrobial tests on *E.coli* and *S.aureus* saponins show inhibition on the growth curve [19]. Also extract of *Sapindus mukorossi* exhibits a strong growth inhibition against the pathogenic yeast *Candida albicans* [17]. Our results are in accordance with the finding reported earlier in the literature.

IV. CONCLUSION

Extraction of *Sapindus mukorossi* with different extraction solvents at different solid-liquid ratio was investigated. Ethanol water solution (50 % v/v) with 1:10 solid- liquid ratio was the best condition for an efficient extraction of saponins. It has higher results from both pure water and ethanol which prove the importance of the extraction process optimization based on extraction solvent type. As seen from HPLC results polarity of solvent is crucial for isolation of desired saponin structures.

Antimicrobial properties of saponin were shown using minimum inhibition concentration values. Although the microorganisms were different from each other antimicrobial properties of saponin exhibited inhibition on the growth curve of microorganism.

REFERENCES

[1] Ö. Güçlü-Üntündağ, and G. Mazza, "Saponins: Properties, applications and processing", Critical Reviews in Food Science and Nutrition, 2007, 47, 231–258.

[2] K. Hostettmann, and A. Marston, "Chemistry and pharmacology of natural products—Saponins", Cambridge: Cambridge University Press. 1995

[3] H. Huang -C., Liao, S. -C., Kuo, Y. -H., Chang, F. -R., & Wu, Y. -C. "Molluscicidal saponins from Sapindus mukorossi, inhibitory agents of golden apple snails, Pomacea canaliculata", Journal of Agricultural and Food Chemistry, 2003, 51, 4916–4919.

[4] J. Augustin, M., Kuzina, V., Anderson, S. B., & Bak, S. "Molecular activities, biosynthesis and evolution of triterpenoid saponins", Phytochemistry, 2011, 72, 435–457.

[5] E. Wina, S. Muetzel, and, K. Becker "The impact of saponins or saponin-containing plant materials on ruminant production"—A review, Journal of Agricultural and Food Chemistry, 2005, 53, 8093–8105.

[6] S. Hassan, M. Haq, A. U. Byrd, J. A. Berhow, M. A. Cartwright, A. L., & C. A. Bailey, "Haemolytic and antimicrobial activities of saponin-rich extracts from guar meal", Food Chemistry, 2010, 119, 600–605.

[7] A. Mostafa, J. Sudisha, M. El-Sayed, S. -I. Ito, N. Yamauchi, M. Shigyo, et al. "Aginoside saponin, a potent antifungal compound, and secondary metabolite analyses from Allium nigrum L", Phytochemistry Letters, 2013, 6, 274–280.

[8] H. Sun, L. Chen, J. Wang, K. Wang, and J. Zhou, "Structure–function relationship of the saponins from the roots of Platycodon grandiflorum for hemolytic and adjuvant activity", International Immunopharmacology, 2011, 11, 2047–2056.

[9] S. Verza, G. Silveira, F. Cibulski, S. Kaiser, S. Ferreira, F. Gosmann, G. et al. "Immunoadjuvant activity, toxicity assays, and determination by UPLC/Q-TOF-MS of triterpenic saponins from Chenopodium quinoa seeds", Journal of Agricultural and Food Chemistry, 2012, 60, 3113–3118.

[10] T. Cheng, C. Lu, J. -F. Wang, J. -S. Lin, L. -J. Kuo, H. -I. and B. -H. Chen, "Antiproliferation effect and apoptosis mechanism of prostate cancer cell PC-3 by flavonoids and saponins prepared from Gynostemma pentaphyllum", Journal of Agricultural and Food Chemistry, 2011, 59, 11319–11329.

[11] B. Joseph, and D. Jini, "Antidiabetic effects of Momordica charantia (bitter melon) and its medicinal potency", Asia Pacific Journal of Tropical Disease, 2013, 3, 93–102.

[12] S. G. Sparg, M. E. Light, and J. van Staden, "Biological activities and distribution of plant saponins", Journal of Ethnopharmacology, 2004, 94, 219–243.

[13] D. T. Piorkowski, and D. J. McClements, "Beverage emulsions: Recent developments in formulation, production, and applications", Food Hydrocolloids, 2013, xxx, 1–37.

[14] Y. Yang, M. E Leser, A. A. Sher, and D. J. McClements, "Formation and stability of emulsions using a natural smallmolecule surfactant: Quillaja saponin (Q-Naturale®)", Food Hydrocolloids, 2013, 30, 589–596.

[15] S. Hiai, H. Oura, and T. Hakajima, "Colour reaction of some sapogenins and saponins with vanillin and sulphuric acid", Planta Medica, 1976, 29, 116–122.

[16] D. Saxena, R. Pal, A. K. Dwivedi and S. Singh "Characterisation of sapindosides in Sapindus mukorosii saponin (reetha saponin) and quantitative determination of sapindoside", B. Journal of Scientific & Industrial Research, 2004, 63(2): 181–186

[17] A. Upadhyay and D.K. Sıngh, "Pharmacologıcal Effects of Sapindus Mukorossi", Rev. Inst. Med. Trop. Sao Paulo 54(5):273-280, September-October, 2012

[18] J. M. Andrews, "Determination of minimum inhibitory concentrations", Journal of Antimicrobial Chemotherapy, 2001, 48, 5-16

[19] M. Ibrahim, A. A Khan, S. K Tiwari, M. A. Habeeb, M.N. Khaja, C.M. Habibullah, "Antimicrobial activity of Sapindus mukorossi and Rheum emodi extracts against H pylori: In vitro and in vivo studies", World J Gastroenterol 2006 November 28; 12(44): 7136-7142

Merve Deniz Köse has a BS degree in Chemical Engineering from Haccettepe University. Currently MS student at the Department of Chemical Engineering in Ege Univeristy. Her research interest is isolation and encapsulation of plant-derived natural compounds.

Prof. Dr. Oguz Bayraktar received his MSc. and Ph.D. degrees in Chemical Engineering from West Virginia University, USA. He worked as a faculty member in İzmir Institute of Technology between 2001-2014. Now, he has been working as a professor at the Department of Chemical Engineering in Ege University. In 2006, he has established a company (Natural Products R & D Ltd. Co.) at İzmir Technology Development Zone. He published more than 30 publications, and 40 conference proceedings. He has 200 citations and his h-index is 7. His research interests include: biopolymers, bioactivities of natural compounds derived from plants, drug delivery systems and nanotechnology.