Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env.Pharmacol. Life Sci., Vol 11[10] September 2022 : 130-137

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Molecular Characterization of antibiogram activities and Bioactive Compound Saponin from the root of *Plectranthus barbatus*

Kabilraj [1], Uthra Selvaraj [2], and Asha Monica[3]
1Department of Biotechnology, Anna University, Chennai, TN
2Research Scholar, CAS in Marine Biology, Annamalai university
3Assistant professor, Department of Biotechnology, St. Joseph's College (Autonomous), Bharathidasan University Affiliated.
E-mail ID: ashamonica7@gmail.com

ABSTRACT
Herbal medicine is also called phytomedicine or phytotherapy. Ayurveda and plant-based remedies for herbal care through day-to-day life experiences are part of the cultural heritage in India. In practically every one of the conventional frameworks of medication, restorative plants assume a significant part and establish their backbone. The aim of the present study is to identify plants using the chloroplast ribosomal protein 16 gene was considered in this study based on molecular identification and itpromotes potential new herbal extracts for antimicrobial and anti-properties at a low cost. Plectranthus barbatus Andr. (Syn. Coleus forskohlii Briq.) is a perpetual spice, having a place with the family Lamiaceae.The saponin from the root of Coleus forskohlii tested has shown better inhibition of antimicrobial properties. This study reports the antimicrobial properties of saponin from the root of Coleus forskohlii.
KEYWORDS: Saponin, antimicrobial, *Coleus forskohlii,* and molecular characterization.

Received 11 .08.2022 Revised 21.08.2022 Accepted 21.09.2022

INTRODUCTION

Medicinal Plant is excellent for the health of individuals and communities [1]. The restorative worth of plants lies in a few compound dynamic substances that produce characterize physiological activity on the human body [2] [3].Secondary metabolites of phytochemicals from plants have important pharmacological activities [4] [5]. Saponins are a course of chemical compounds which is found in different plant species [6] [7]. More particularly, they are amphipathic glycosides gathered phenomenologically by the soap-like froth they create when shaken in fluid arrangements, [8] and basically by having one or more hydrophilic glycoside moieties combined with a lipophilic triterpene or steroid derivative [9] [10].

Saponins characteristically have a sharp taste and a few are known to be poisonous. The number of saccharide chains connected to the sapogenins (aglycone) can shift as can the length of each chain [11] [12]. The saccharide chain length, so distant, changes from 1 to 11 sugar residues, with the numbers 2–5 being most habitually experienced with both straight and branched chains being spoken [13] [14]. All saponins have a connection of at slightest one sugar chain to the aglycone and can be depicted as mono, di, or tridesmosidic depending on the number of saccharide chains join to the aglycone.Pharmaceutical industries buy saponins in large quantities because of their use for the semi-synthesis of steroidal drugs for Phyto-therapy and in the cosmetic industry [15]. The antimicrobial activity of increasing global trend of resistance to drugs among Gram-positive and Gram-negative bacteria pose major challenges to health care workers [16] [17]. Multidrug-safe microscopic organisms are impervious to a few unique anti-infection agents [18] [19]. The administration of multi-drug safe bacterial strains is troublesome on the grounds that treatment choices are restricted and assuming accessibility are past the scope of poor people [20]. This might expand dangers of death, increment length, and the expense of hospitalization, and increment the expense on medical services frameworks [21]. There is a pressing need to investigate new viable regions for the treatment of irresistible infections [22].

This study aimed to identify plants using chloroplast gene and separate naturally occurring secondary metabolites Saponins from *Plectranthus barbatus* extracted compounds and evaluate their antimicrobial against bacterial, fungal pathogens.

MATERIAL AND METHODS

Collection and Identification of plants:

*Plectranthus barbatus*used in the study was collected and identified using molecular characterization. *Plectranthus barbatus*was collected randomly from the region of in and around Kolli hills. *Plectranthus barbatus*was air-dried and then homogenized to a fine powder and stored in an airtight bottle. The extracts were then, dried in a vacuum and stored in a refrigerator. The yield of extract 50grams of whole root powder yielded 11.6g.

Extracts Preparation:

Several extracts (methanol, benzene, chloroform, petroleum ether, ethanol, and water) of the ethanolic extract of the plant of *Plectranthus barbatus*were probed for their phytochemical analysis.

Isolation, separation, and purification of saponin from *Plectranthus barbatus*roots

Powdered plant material is extracted by successive solvent treatment in a Soxhlet extractor. The first powdered material is defatted with petroleum ether or n-hexane. Defatted material is then extracted with methanol. The methanolic extract is concentrated using suitable means (preferably under vacuum by rotary evaporation), producing dry extract. Dried methanol extract suspended into distilled water and shaken with n butanol**,** followed by precipitation of crude saponins mixture by addition of solvent ether.

Estimation of saponins

20 g of sample is put into a conical flask and 100 ml of 20% aqueous ethanol is added. The above solution is heated on a bather bath for 4 h with continuous stirring at about 55°C. The mixture is filtered and the residue is again extracted with 200 ml 20% ethanol. The combined extracts are concentrated to about 40 ml over a water bath at about 90°C. The pack is moved into a 250 ml isolating pipe and 20 ml of diethyl ether is added and shaken enthusiastically. The watery layer is recuperated while the ether layer is disposed of. The purification process is repeated. 60 ml of n-butanol is added. The combined n-butanol extracts are washed twice with 10 ml of 5% aqueous sodium chloride. The remaining arrangement is warmed in a water bath. After vanishing, the examples are dried on the stove to a steady weight and the saponin content is determined as a rate

Thin layer chromatography

Saponins are generally present in plants as glycosides. Powdered drug is extracted by heating under reflux with 70% alcohol. The filtrate is evaporated and a concentrated solution is used for TLC. To enrich the above-concentrated solution in saponins, it is shaken with water saturated-n butanol. Finally,the organic layer is separated, concentrated to get a fraction rich in saponins. The most commonly employed solvent systems are Chloroform- glacial acetic acid methanol-water (60:32:12:8) and ethyl acetate formic acid-glacial acetic acid-water (100:11:11:26).

Antimicrobial activity

The antimicrobial activity was tested against saponin from *Plectranthus barbatus.*The inoculation of microorganisms was prepared from the bacterial culture [23]. About 15-20 ml of Muller - Hinton agar medium was poured in the sterilized Petri dish and allowed for solidification. One drop of bacterial strains was spread over the medium by a rod.Wells of 6nm in diameter and about 2cm a part punctured in the culture medium using sterile cork borers. The solution of saponin from *Plectranthus barbatus*(10mg/ml) was prepared in sterile distilled water. 100 ml of the extracts were transferred into holes using sterile Pasteur pipettes. Plates were incubated in the air at 37^{o}C for 24 hours. Antimicrobial exercises were assessed by estimating hindrance zone measurements.

RESULTS AND DISCUSSION

In the present study, different extracts of Plectranthus barbatushave been investigated for their phytochemical screening. From methanol extracts synthesis, isolation, and purification of saponin from *Plectranthus barbatus*and its *antimicrobial*activity. Phytochemicals protect us against many diet-related diseases. Thecommonly known phytochemical compounds from *Plectranthus barbatus* are acridonealkaloids, coumarins, volatile substances, terpenoids, flavonoids, andfuroquinolines [24].

Molecular Characterization:

Plectranthus barbatus plants were identified using molecular characterization with the help of sequencing for that first isolated genomic DNA from *Plectranthus barbatus* using CTAB method and amplify with the help *rps16* gene and sequenced in ABI big dye cycle termination reaction the sequence was BLAST to identify closely related species and submitted sequenced in NCBI and accession number of our Sequence is OM969821.

Phytochemical analysis:

The qualitative data presented in table 1, *Plectranthus barbatus*reveals the presence of a wide range of secondary components alkaloids, flavonoids, phenols, tannins, Saponin, and steroids. The petroleum ether extract showed the presence of alkaloids, saponin, tannin, and flavonoids. The *Plectranthus barbatus*methanolic extract showed the presence of most of the bioactive compounds like alkaloids, flavonoids, Saponin, triterpenoid, steroid, carbohydrates, and tannins. Alkaloid, flavonoid, saponin, triterpenoid, steroid, and tannin were present in the aqueous extract of *Plectranthus barbatus.* From the above results, it was inferred that a broad range of secondary metabolites was present in the methanol extract of *Plectranthus barbatus.* Hence, it is utilized for further experimental analysis.Medicinal plants have potent phytochemical components which are important sources of antibiotic compounds and are responsible for the therapeutic properties [25].

Synthesis, Isolation, and Purification of Saponin from *Plectranthus barbatus*

Saponin isa phytochemical, found mainly but not exclusively in plants,which exhibit foaming characteristics and consist of polycyclic aglycones attached to one or more sugar side chains. The aglycone part, which is likewise called a sapogenin, is either a steroid (C27) or a triterpene (C30). The frothing capacity of Saponin is brought about by the mix of a hydrophobic (fat-solvent) sapogenin and a hydrophilic (water-dissolvable) sugar part. Extraction of Saponin involved hot extraction of the plant material using aqueous-alcoholic solutions followed by evaporation of the alcohol and then extraction of Saponin into butanol by liquid-liquid extraction. The problem with hot extractions is that labile functionalities (e.g.,acylated forms) may disintegrate to produce artifacts rather than genuine Saponin. Furthermore, extraction with methanol (MeOH) especially for steroidal Saponin may result in the formation of methyl derivatives not originally found in the plant. Thus, to obtain the real composition of Saponin, cold extractions with methanol-water solutions would be better. It should be noted that in liquid-liquid extractions, some highly polar Saponin, such as bidesmosides and tridesmosides, may all remain in the aqueous layer or the extraction may not be quantitative. The fact that Saponin occurs in plants as a mixture of structurally similar compounds of similar polarity renders a challenge when it comes to separating. Saponin is also important therapeutically as they are shown to have hypolipidemic and anticancer activity. Saponin is also necessary for the activity of cardiac glycosides. The two significant sorts of steroidal sapogenin are diosgenin and hecogenin. Steroidal Saponin is used in the commercial production of sex hormones for clinical use. For example, progesterone is derived from diosgenin. The most plentiful beginning material for the amalgamation of progesterone is diosgenin secluded from Dioscorea species, previously provided from Mexico, and presently from China. Other steroidal chemicals, for example cortisone and hydrocortisone, can be ready from the beginning material hecogenin, which can be segregated from Sisal leaves tracked down broadly in East Africa[26].

Recovery of Saponins from Deemulsified Emulsion

The dried methanol extract of *Plectranthus barbatus* acted as a surfactant/emulsifier by enhancing the emulsification (i.e. solubilization) of ethyl-ether in water. The recovery of Saponin was possible in a relatively pure state after the emulsion has been destabilized. Saponin materials were deposited at the middle and bottom layers of destabilized emulsion, in varieties of shapes. DE emulsification was supported by phase-separation, which is secondary to phase inversion from oil-in-water (o/w) to water-in-oil (w/o) emulsion.

The kind of detachment noticed, portrayed Winsor II stage harmony, by which the emulsifiers substances are immersed in the upper natural stage, in seemingly rich suspension and exceptionally turbid. There was observable sedimentation of particles in the upper phase, which further enhanced the recovery of Saponin. The purity of the finished product was labeled and tremendously improved in three successive purification cycles, likewise the physical appearance [27] . Ethyl ether behaved as a suitable anti-solvent medium which promoted the precipitation and sedimentation of particles in the upper organic phase as shown in Figure: 2.

Different methods have been reported for the extraction of crude Saponin and pure diosgenin from *Plectranthus barbatus*, Separation and extraction of diosgenin can be carried out by thin-layer chromatography (TLC) or high-performance thin-layer chromatography (HPTLC), whereas, in other methods, it can be extracted directly in pure form. In the method diosgenin was extracted from cultured cells of Dioscoreazingiberensis.[28] it has been revealed contain the greatest total amount of Saponin accumulation. The yam tuber cortex has been discovered to possess the highest amount of Saponin from 582.53 µg/g dw which was about 2.55 times higher than tuber flesh of 227.86 µg/g dw [29]. However, the total saponin concentration has been reported to contain the highest level in leaves from the four varieties of Switchgrass [30][31]and greenhouse-grown Maesalanceolata.

Antimicrobial Activity

Results of antifungal activity showed almost similar trends as antibacterial activity. The results of this study revealed that the diameter of the zone of inhibition for fungal strains was less than the diameter measured for bacterial strains. Saponin showed a greater inhibitory effect on bacterial strains as compared to fungal strains. This distinction is due to differences in the cell wall structure and protein synthesis of fungal and bacterial strains. These findings are in agreement with the observations of many other researchers [32].

Table 1: Qualitative Estimation of Phytochemical Constituents of Different Extracts of *Plectranthus barbatus*

+++ = high; ++ = Moderate; + = low; ND = not detectable

S.No	Bioactive compounds	Aqueous	Methanol	Benzene	Chloroform
1	Alkaloid	+	++	ND	ND
2	Flavonoid	++	+++	ND	+
3	Triterpenoid	++	+++	++	ND
4	Carbohydrates	ND	++	ND	ND
5	Saponin	+++	++	ND	ND
6	Steroids	+	++	++	++
7	Amino acid	+	++	ND	ND
8	Tannin	++	+++	ND	ND
9	Gums & Mucilage	ND	ND	ND	ND
10	Chlorogenic compound	+	++	ND	ND

Table 2: Effect of antimicrobial activities of saponin from *plectranthus barbatus*

S.NO	MICROORGANICS	C	100µl	75 µl	50 µl	25 µl
GRAM POSITIVE BACTERIA						
1	*Staphylococcus aureus*	22	17	15	13	9
2	*Enterococccusfaecalis*	20	18	14	11	8
3	*Bacillus subtillis*	23	16	13	12	10
4	*Micrococcus luteus*	25	20	18	15	13
GRAM NEGATIVE BACTERIA						
1	*E.coli*	24	18	15	12	10
2	*Klebsiella pneumonia*	26	20	17	15	12
3	*Salmonella typhi*	23	17	13	11	9
4	*Proteus mirabilis*	19	14	11	9	7
FUNGAL						
1	*Candida albicans*	20	15	13	11	8
2	*Aspergillus flavus*	18	12	10	9	7

Antibacterial effects of the Saponin from *Plectranthus barbatus*, against totally eight bacterial strains and two fungal strains were used throughout the investigation. The bacteria used were *Bacillus subtillis, Staphylococcus aureus, Enterococccus faecalis, Salmonella typhi, Shigellafluxneri, Sterptococcusepidermitis, E.coli, and Psuedomonus aerogenosa*. The parasitic strains utilized were Aspergillus Niger and Candida albicans. Suggest that they may possess remarkable therapeutic action in the treatment of gastrointestinal tract and skin diseases and other infectious diseases.

Nowadays phyto pharmacological investigation has created a new field to discovery plant derivative drugs, which are effective in remedial of certain diseases, and renewed the attention in herbal medicines. It is estimated that about 30% of the pharmaceuticals are prepared from plants derivatives [33].

The antibacterial activity of the tested extract of Saponin from *Plectranthus barbatus*, showed a significant reduction in bacterial growth in terms of zone of inhibition. The hydro alcohol extract showed dose-dependent activity i.e. while increasing in the concentration of extract, the zone of inhibition is also increased. The previous study showed maximum growth of inhibition (30 mm) was observed in Saponin from *Plectranthus barbatus*[34].

Fig. 1: Phytochemical screening analysis of a Methanolic extract of *Plectranthus barbatus*

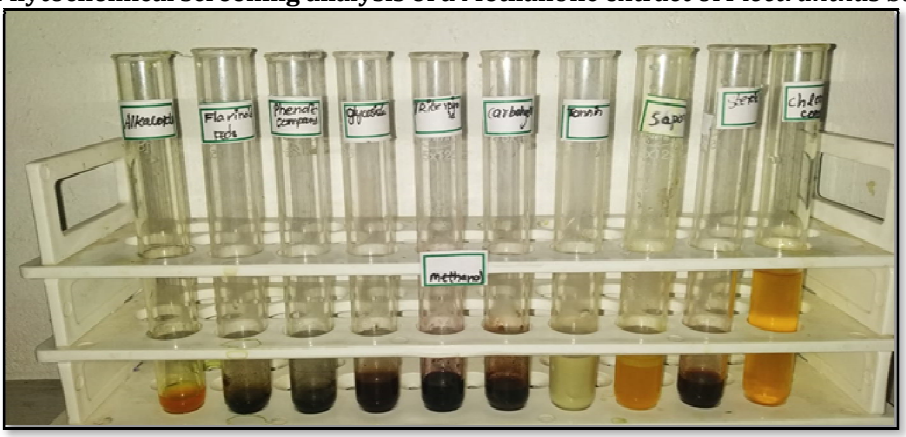


Fig. 2: Confirmation test for saponin froth test

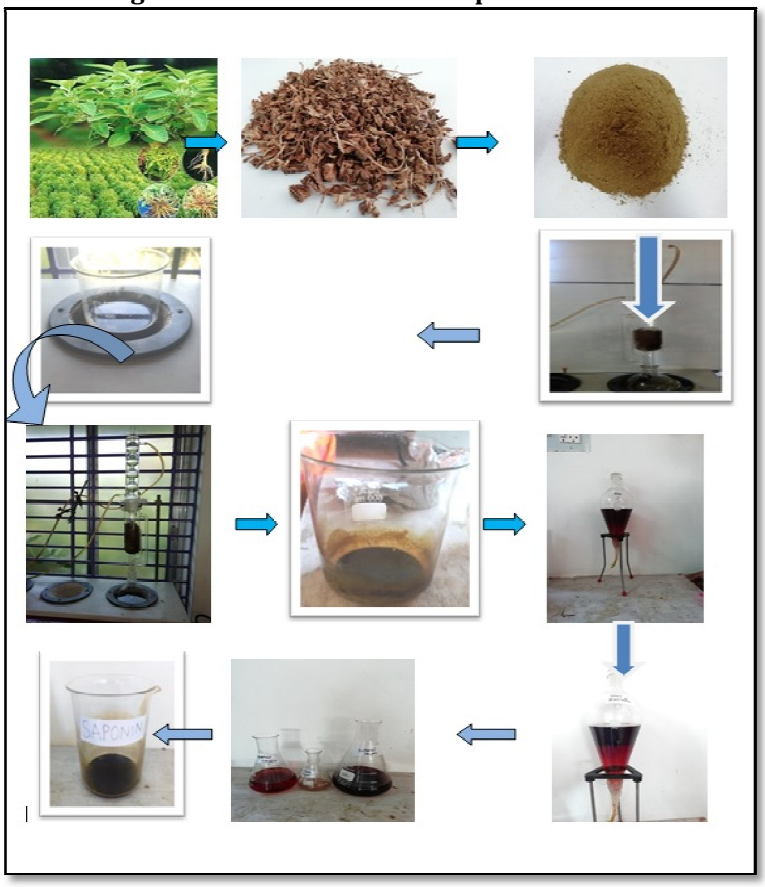
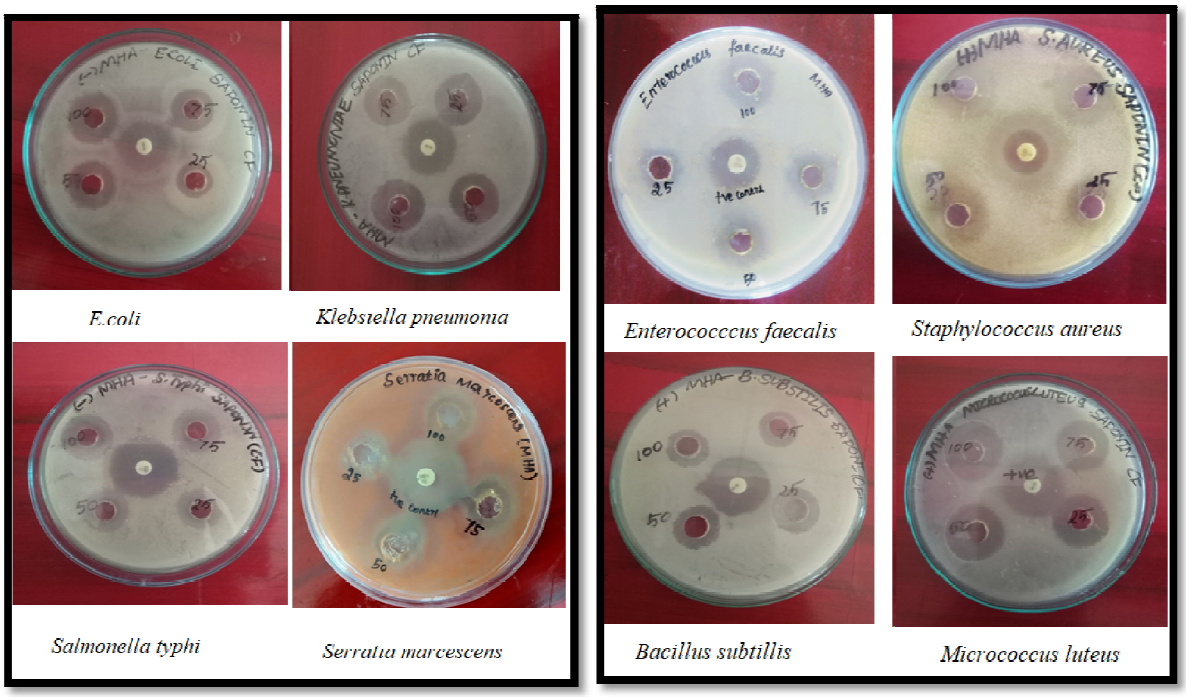
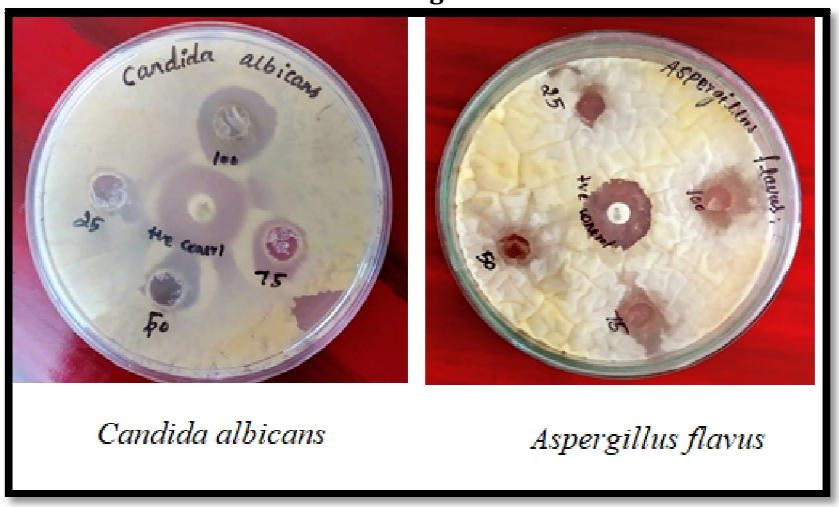


Fig. 3:Diagrammatic representation of effect of antimicrobial activities of Saponin from *Plectranthus barbatus*



Gram Positive Bacteria **Gram Negative Bacteria**

Fungal



CONCLUSIONS

Phytochemical screening of the plant extract identified flavonoids and phenolic type compounds as the major antioxidant and anticancer potent in the *Plectranthus barbatus*. Phytochemical screening analysis exhibited the presence of a wide range of secondary metabolite namely Flavonoids, Alkaloids, Terpenoids, Steroids, Saponins. In the present study, separation and purification of saponins from *Plectranthus barbatus*, revealed for the presence of saponins in the plant material may be confirmed using dry or wet test depending on the foam formation characteristics. Determination of total saponins contents can be proceeding *via* consecutive solvent extraction by n-butanol.the saponins from *Plectranthus barbatus*, tested for antimicrobial activity against the tested pathogens. The findings of the present investigation suggest that the *Plectranthus barbatus*, possess compounds with antimicrobial activity and could serve as useful sources for new antimicrobial agents. The present study revealed that the methanol extract of *Plectranthus barbatus*of phytochemical analysis proves the presence of numerous active compounds responsible for antimicrobial activities and justifies the medicinal uses. Hence, *Plectranthus barbatus* root

might be utilized for finding new drugs, and further investigation needs to elute novel bioactive compounds and toxicity profiles through *in vitro* and *in vivo* models.

ACKNOWLEDGEMENT
The authors sincerely thank Anna University, Annamalai University, and Bharathidasan University.

Conflict of interest statement:
We declare that we have no conflict of interest.

REFERENCES

1. Tungmunnithum, D., Thongboonyou, A., Pholboon, A., & Yangsabai, A. (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines*, *5*(3), 93.
2. Hussein, R. A., & El-Anssary, A. A. (2019). Plants secondary metabolites: the key drivers of the pharmacological actions of medicinal plants. *Herbal medicine*, *1*, 13.
3. Kennedy, D. O., & Wightman, E. L. (2011). Herbal extracts and phytochemicals: plant secondary metabolites and the enhancement of human brain function. *Advances in Nutrition*, *2*(1), 32-50.
4. Mendoza, N., & Silva, E. M. E. (2018). Introduction to phytochemicals: secondary metabolites from plants with active principles for pharmacological importance. *Phytochemicals: Source of antioxidants and role in disease prevention*, *25*.
5. Bhuiyan, F. R., Howlader, S., Raihan, T., & Hasan, M. (2020). Plants metabolites: possibility of natural therapeutics against the COVID-19 pandemic. *Frontiers in Medicine*, 444.
6. El Aziz, M. M. A., Ashour, A. S., & Melad, A. S. G. (2019). A review on saponins from medicinal plants: chemistry, isolation, and determination. *J. Nanomed. Res*, *8*(1), 282-288.
7. Kregiel, D., Berlowska, J., Witonska, I., Antolak, H., Proestos, C., Babic, M., ... & Zhang, B. (2017). Saponin-based, biological-active surfactants from plants. *Application and characterization of surfactants*, *6*(1), 184-205.
8. Honda, T. J., Yelwa, J. M., Amos, C., & Mshelia, A. D. International Journal of Advanced Chemistry Research.
9. Moses, T., Papadopoulou, K. K., & Osbourn, A. (2014). Metabolic and functional diversity of saponins, biosynthetic intermediates and semi-synthetic derivatives. *Critical reviews in biochemistry and molecular biology*, *49*(6), 439-462.
10. Abdelrahman, M., & Jogaiah, S. (2020). Metabolic and Functional Diversity of Saponins. In *Bioactive Molecules in Plant Defense* (pp. 15-35). Springer, Cham.
11. Durán, A. G., Celaj, O., Macías, F. A., & Simonet, A. M. (2021). Dereplication of Bioactive Spirostane Saponins from Agave macroacantha. *Journal of natural products*, *84*(11), 2904-2913.
12. García Durán, A., Celaj, O., Macías Domínguez, F. A., & Simonet Morales, A. M. (2021). Dereplication of Bioactive Spirostane Saponins from Agave macroacantha.
13. Waller, G. R., & Yamasaki, K. (Eds.). (2012). *Saponins used in food and agriculture* (Vol. 405). Springer Science & Business Media.
14. Waller, G. R., & Yamasaki, K. (Eds.). (2013). *Saponins used in traditional and modern medicine* (Vol. 404). Springer Science & Business Media.
15. Güçlü-Üstündağ, Ö., & Mazza, G. (2007). Saponins: properties, applications and processing. *Critical reviews in food science and nutrition*, *47*(3), 231-258.
16. Avato, P., Bucci, R., Tava, A., Vitali, C., Rosato, A., Bialy, Z., & Jurzysta, M. (2006). Antimicrobial activity of saponins from Medicago sp.: structure-activity relationship. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *20*(6), 454-457.
17. Shi, Z. Y., Zeng, J. Z., & Wong, A. S. T. (2019). Chemical structures and pharmacological profiles of ginseng saponins. *Molecules*, *24*(13), 2443.
18. Laxminarayan, R., Bhutta, Z., Duse, A., Jenkins, P., O'Brien, T., Okeke, I. N., ... & Klugman, K. P. (2011). Drug resistance.
19. Laxminarayan, R., Bhutta, Z. A., Duse, A., Jenkins, P., O'Brien, T., Okeke, I. N., ... & Klugman, K. P. (2006). RISK FACTORS. *Drug Resistance*, 101.
20. Nikaido, H. (2009). Multidrug resistance in bacteria. *Annual review of biochemistry*, *78*, 119-146.
21. Abdelrahman, M., & Jogaiah, S. (2020). Metabolic and Functional Diversity of Saponins. In *Bioactive Molecules in Plant Defense* (pp. 15-35). Springer, Cham.
22. Aslam, B., Wang, W., Arshad, M. I., Khurshid, M., Muzammil, S., Rasool, M. H., ... & Baloch, Z. (2018). Antibiotic resistance: a rundown of a global crisis. *Infection and drug resistance*, *11*, 1645.
23. Laxminarayan, R., Bhutta, Z., Duse, A., Jenkins, P., O'Brien, T., Okeke, I. N., ... & Klugman, K. P. (2011). Drug resistance.
24. Forte, B., Ottilie, S., Plater, A., Campo, B., Dechering, K. J., Gamo, F. J., ... & Gilbert, I. H. (2021). Prioritization of Molecular Targets for Antimalarial Drug Discovery. *ACS infectious diseases*, *7*(10), 2764-2776.
25. Asha Monica, A., & SR, S. (2020). Green synthesis and characterization of silver nanoparticles from Ocimum basilicum and their antimicrobial antioxidant and anticancer activity.
26. Mujeeb, F., Bajpai, P., & Pathak, N. (2014). Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of Aegle marmelos. *BioMed research international*, *2014*.
27. Shad, A. A., Ahmad, S., Ullah, R., AbdEl-Salam, N. M., Fouad, H., Rehman, N. U., ... & Saeed, W. (2014). Phytochemical and biological activities of four wild medicinal plants. *The Scientific World Journal*, *2014*.

28. Shen, L., Xu, J., Luo, L., Hu, H., Meng, X., Li, X., & Chen, S. (2018). Predicting the potential global distribution of diosgenin-contained Dioscorea species. *Chinese medicine*, *13*(1), 1-10.
29. Cheok, C. Y., Salman, H. A. K., & Sulaiman, R. (2014). Extraction and quantification of saponins: A review. *Food Research International*, *59*, 16-40.
30. Deshpande, H. A., & Bhalsing, S. R. (2014). Isolation and characterization of diosgenin from in vitro cultured tissues of Helicteres isora L. *Physiology and molecular biology of plants*, *20*(1), 89-94.
31. Moses, T., Papadopoulou, K. K., & Osbourn, A. (2014). Metabolic and functional diversity of saponins, biosynthetic intermediates and semi-synthetic derivatives. *Critical reviews in biochemistry and molecular biology*, *49*(6), 439-462.
32. Danish, P., Ali, Q., Hafeez, M. M., & Malik, A. (2020). Antifungal and antibacterial activity of aloe vera plant extract. *Biological and Clinical Sciences Research Journal*, *2020*(1).
33. Veeresham, C. (2012). Natural products derived from plants as a source of drugs. *Journal of advanced pharmaceutical technology & research*, *3*(4), 200.
34. Dahiya, P., & Purkayastha, S. (2012). Phytochemical screening and antimicrobial activity of some medicinal plants against multi-drug resistant bacteria from clinical isolates. *Indian journal of pharmaceutical sciences*, *74*(5), 443.

CITATION OF THIS ARTICLE

Kabilraj, Uthra Selvaraj , and Asha Monica· Molecular Characterization of antibiogram activities and Bioactive Compound Saponin from the root of *Plectranthus barbatus.*Bull. Env. Pharmacol. Life Sci., Vol 11 [10] September 2022: 130-137

Filename: 20
Directory: C:\Users\Acer\Downloads
Template: C:\Users\Acer\AppData\Roaming\Microsoft\Templates\Normal.dotm
Title:
Subject:
Author: mk009
Keywords:
Comments:
Creation Date: 10/23/2022 10:31:00 PM
Change Number: 4
Last Saved On: 3/31/2023 9:35:00 AM
Last Saved By: Windows User
Total Editing Time: 14 Minutes
Last Printed On: 3/31/2023 9:35:00 AM
As of Last Complete Printing
Number of Pages: 8
Number of Words: 3,717 (approx.)
Number of Characters: 21,191 (approx.)