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Original Article

Assessments of Antibacterial Potential of *Commiphoramukul* (*Guggulu* Extract)

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ABSTRACT

The *Commiphoramukul* (guggul gum) is extracted from the stem of *C. mukul.* It is important for various medicinal and pharmacological applications used in various formulation of ayurveda. Guggul gum is pharmacologically active in controlling rheumatoid arthritis, obesity and peptic ulcers. The pharmacological and clinical studies on its crude drug constituents and various extractives have revealed its significant hypocholestermic, hypolipidimic, antiinflammatory, antirheumatic and antifertility activities The potential antibacterial efficacy of guggul gum was checked against four Gram-positive and four Gram-negative bacterial strains *Streptococcus agalactiae*,*Staphylococcusaureus*.*Escherichia Coli* and *Pseudomonasaeruginosa.* The antibacterial activity was assessed by agar well diffusion and two fold serial broth dilution methods. Gram-positive bacterial strains were found to be the most susceptible organisms compare to Gram-negative towards guggul gum extracts. In traditional medicine and Ayurveda plants have been known to relieve various diseases. The medicinal properties of plants mainly depend on secondary metabolites that are playing key role I relive from various diseases. In the present study ethyl acetate and methanol extract of *C.mukul*were screened for the presence of phytochemicals by standard procedures. Phytochemical analysis revealed that the presence or absence of alkaloids, flavonoids, proteins, quinons, reducing sugars, saponin, and phenolics. Antimicrobial activity was observed in *C.mukul* against *E.coli*,*S.agalactiae* and*S. aureus*which may be due to different bioactive compounds present in the plant of *C.mukul.* In general very less or no activity is observed against *P. aeruginosa*

Key words: *Commiphoramukul*, guggul gum, antibacterial activity

1. INTRODUCTION

Traditionally oleo gum resin is collected by the tapping of oleo gum-resin from erect type guggul plants in summer and the yield is about 200-800 g per plant. The plant contains essential oils, mainly myresene, dimyrecene and polymyrecene, Z-guggulosterone, E-guggulosterone [1]. *C.mukul*plant is one of the most exploited plant for gum and resin production due to the plant is under Red data list of

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IUCN and various approaches have been made by various organizations to conserve this species of plant by means of plant tissue culture methods [2].

Medicinal plants are the richest source of drug for traditional system of medicines, modern medicines, food supplements, and chemical entities for synthetic drugs. Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involves the use of plant extracts [3]. Medicinal plants contain some organic compounds, which provide physiological action on the human body, that includes alkaloids, flavonoids, tannins, steroids [4]. These bioactive compounds are generally derived from leave, root, stem, barks [5]. Presence of guggul is mainly confirmed by tapping the bark of *C.mukul* where 200-300 g of dry resin is present which is used in treatment of various diseases such as obesity, hypercholesteremia and other disorders related to lipid metabolism [6]. *C. mukul* plant produces guggulosterone that have the capability to stimulate Low density lipid cholesterol (LDL) receptor binding activity in hepatocytes and enhances its catabolism, and also inhibits oxidative modification of LDLcholesterol [7] Guggulsterone – Z and E, the active constitute of resin are responsible for lipid lowering properties in human blood [8, 9, 10] .Despite having so many activities no reports found on the antibacterial activity of guggul gum up to structural identification of active compound. So in this context, present study was under taken to study the antibacterial activity and to identify antibacterial compounds present in guggul gum.

2. METHODOLOGY

Phytochemical analysis

Phytochemical tests were carried out on the methanol & ethyl acetate extracts of C.*mukul*using standard procedure to identify and to confirm the constituents present in these extracts. [11]

Test for Alkaloids

Mayer's test was used to determined alkaloids, 50µl of both methanol and ethyl acetate extracts of *C.mukul* were treated with 1.36g of mercuric chloride and 5g of potassium iodide in 100ml distilled water/Mayer's reagent and studied for the formation of cream colour precipitate. Wagner's reagent: 10-50µl of extract was treated with 1.27g of iodine and 2g of potassium iodide in 100ml distilled water.

Test for flavonoids

NaOH and HCL test was used to detect flavonoids Small amount of guggulu extract was treated with aqueous NaOH and HCL and observed for the formation of yellow orange colour. H_2SO_4 test: Extract was treated with conc. H_2SO_4 and observed for the formation of orange colour for the presence of flavonoids.

Test for proteins

Millon's test was detect proteins 2ml of Millon's reagent was mixed with plant extract and observed for the formation of white color which turns red on gentle boiling that confirms the presence of protein.

Test for tannins

Extract was treated with 10% lead acetate and observed for the formation of white color precipitate.

Test for reducing sugar and carbohydrate

Fehling's test was carried out Fehling A and Fehling B reagents were mixed together in equal volume and 2ml of it was added to extract and boiled in water bath and cooled. The appearance of brick red precipitate at the bottom of the test tube indicated the presence of reducing sugar. Benedict's test: *C.mukul*plant extracts were mixed with 2ml of Benedict's reagent and boil, and observed for the formation of reddish brown precipitate, which indicates the presence of the carbohydrates

Libermannburchard's test: Extracts were treated with 2-3 ml of acetic anhydride and few drops of glacial acetic acid followed by drops of conc. H_2SO_4 and observed for the formation of bluish green colour that indicates the presence of steroids.

Test for phenolic compounds

By $FeCl_3$: The guggul extracts were treated with neutral ferric chloride solution and observed for the formation of violet colour, indicating the presence of phenolic compounds. By Sodium chloride test. Theguggul extracts were treated with 10% sodium chloride solution and observed for the cream colour.

Test for saponins

1ml of each extracts of guggul were diluted with 20ml of distilled water and shaken well in a test tube and observed for the formation of foam on upper region of the test tube the indicate that the saponnins are present in the extracts of *C.mukul*

Antimicrobial activity testing of the extract against the selected bacterial strains

Preparation of methanol extracts

Extract was prepared by cold extraction method in which 25 g of *guggul* powder was soaked in equal volume of dichloromethane and methanol (125 ml) for 24 h at room tempera-true and was shaken occasionally. The extract was filtered and then concentrated by evaporating the solvent at room temperature. The residue (5 g) was stored in the airtight glass bottle in a refrigerator. Concentrations of extract (100 mg/ml) were prepared in DMSO (Dimethyl Sulfoxide) for checking the antibacterial activity and further identification and characterization of compound [12]. In the present study Gram-positive bacteria *Staphylococcus aureus*, *Streptococusagalactae*) and Gram-negative bacteria (*Escherichia coli*,, *Pseudomonas aeruginosa*, were tested which were obtained from the Department of Microbiology, Faculty of medicine University of Colombo. The bacterial cultures was maintained and grown in nutrient broth and nutrient agar medium. The fresh bacterial cultures compared with 0.5 McFarland turbidity standard, which is equivalent approximately to $1x10^8$ CFU/ml [13] was used for the bioassay.

Antibacterial activity

Bioassay was carried out by agar well diffusion method [14] from each bacterial culture 0.1 ml was spread on the nutrient agar plate. The well of 6 mm diameter punched off with the help of sterile cork borer in the nutrient agar plate and then the volume 100mg/ml of extract was carefully added. Plates were incubated at 4°C for pre-diffusion of extracts in a refrigerator. The plate's were then incubated at 37°C in an incubator for 24 h. The antibacterial activity was evaluated by measuring the diameter of the zone of inhibition excluding the diameter of the well. Amoxicillin at a concentration of 100mg/ml as the positive control and 100% DMSO were used as the negative control.

3. RESULTS

The present study revealed that the *Ksharasutra* contains bioactive compounds. The phytochemical constituents of*Ksharasutra*were screened by qualitative methods and the results are presented in Table1.Accordingly, the brownish green color formation indicates the presence of Tannin. Similarly, the presence or absence of color change indicate positive and negative results (Table 1). The study showed that the positive results were obtained for, alkaloids, flavonoids, saponins and tannins while steroids, reducing sugars, sugars and cardiac glycoside gave negative results. (Table 1)

Table 1: Results of Phytochemical constitution of *Commiphoramukul*

Phytochemical Constituents	**Result Present(+)/Absent(-)**
Steroids	-
Alkaloids	+
flavonoids	+
Tannins	+
Glycoside	-
Saponins	+
Reducing sugar	-
Non reducing sugar	-

C.muku, used as binding agent in preparation of *ksharasutra*as an alternative to the Latex used. It was observed that this *guggulu* resin has potential ZOI for the *S. aureus*, 14.5±0.54, *E.coli*, 9±0.635 and *S. agalactiae*, 10±0.258. In comparison to the standard antibiotic, amoxicillin ZOI was significantly high. **(**Table 2). So *Guggulu*had comparatively low ZOI for the tested bacteria in comparison to the standard antibiotic;amoxicillin

Table 2: ZOI of bacteria for *Comiphoramukulgugguluvs* amoxicillin

Type ofbacteria	Number ofreplicates	Mean±SE for gugul	Mean±SE for Amoxxicillin	t- value	Probability value
S. aureus (ATCC 25923)	3	14.5±0.54	30.23±1.19	12.89	P<0.05 SIG
E.coli (ATCC 25922)	3	9±0.63	21±0.447	23.24	P<0.05 SIG
S.agalactiae ATCC (19615)	3	10±0.25	22.6±0.918	13.27	P<0.05 SIG
P.aeruginosa ATCC (27853)	3	6±0	6±0		

4. DISCUSSION

Resin of *C.mukul (guggul)* has moderate antibacterial activity against all the Gram-positive and Gram-negative bacteria showing its ZOI for *S.aureous*14.5±0.223*S.agalactea*10±0.25 and *E coli,* 9±0.258 and no inhibition was observed for *the P.aerguneosa.* Amoxicillin possess higher ZOI for *S.aureousE. coli and S.agalactea.* The difference was statistically significant (Table 2) this finding comply with the findings of Kalpesh*et al.,* 2010. [11]

The *guggul gum* extract was effectively inhibited the growth of selected the Gram-positive and Gram-negative bacteria. *Guggul*resin also used as a binding agent of *Ksharasutra* perpetration alternative to the *Euphorbia antiquorum*latex since its clinical efficacy already established [15]. Guggulsterone is an aromatic steroidal ketonic compound obtained of *C. mukul*. Owing to its multifarious medicinal and therapeutic values as well as its various other significant bioactivities, guggulsterone has high demand in pharmaceutical, Guggulsterone possess anti-inflammatory, edema inhibitory, fungistatic, fungicidal, antibacterial actions (kinmura*et al.,* 2001) these scientific evidences made it an ideal drug to be included in the preparation of *ksharasutra.*

The results obtained from the current study suggest that extract of *Guggul gum* possesses significant antibacterial activity against Gram-positive bacteria and moderate activity against Gram-negative once. *S. aureous* and *S. aglactae* were found to be most susceptible organisms whereas *E. coli* was shown resistance and no inhibition reported for the *P.aerugunosa*

Antimicrobial susceptibility tests of different *Ksharasutra* extract against Gram-positive and Gram-negative bacteria of both clinical isolates and standard type cultures showed significant differences ZOI which comply with the observation recorded by Negi*et al.,* 1999. [14]

In this study presence of different secondary metabolites like terpenoid, flavanoid, saponin, tannin, protein, alkaloid, glycosides and steroid were confirmed by various phytochemical tests for methanol and ethyl acetate extracts. These showed that the extracts are rich in most of the secondary metabolites. These bioactive compounds were reported to show medicinal activity as well as physiological activity. Some of *C.mukul*plant metabolites were found to be absent in some of the extracts analysed. In the present study confirmed that phenolic compounds like tannins present in the cells of plants are potent inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plant pathogens (Kamba *et al.,* 2010).

Antimicrobial activity was observed against *E.coli*,*S.agalactiae* and*S. aureus*which may be due to different bioactive compounds present in the plant of *C.mukul*. Generally no activity is observed against *P.aeruginosa*

5. CONCLUSION

The results obtained from the current study suggest that extract of*C.mukul* possesses significant antibacterial activity against Gram-positive bacteria and moderate activity against Gram-negative bacteria. *E.coli, S.agalactiae* and *S. aureus* were found to be most susceptible organisms whereas *P. aeruginosa* was shown resistance

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Conflict of Interest: None

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