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"A REVIEW ON ANTIBACTERIAL ACTIVITY OF HIBISCUS ROSA SINENSIS AGAINST ENTERIC PATHOGENS"

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ABSTRACT

Introduction: *Hibiscus rosa sinensis* is known as China rose belonging to the Malvaceae family. This plant has various important medicinal uses for treating wounds, inflammation, fever and coughs, diabetes, infections caused by bacteria and fungi, hair loss, and gastric ulcers in several tropical countries.

Methodology: In this review article, the antibacterial activity was evaluated by using disc and agar diffusion methods. Experiment from recent studies showed that various types of extracts from all *H. rosa sinensis* parts exhibited a wide range of beneficial effects such as hypotensive, anti-pyritic, anti-inflammatory, anti-cancer, antioxidant, anti-bacterial, anti-diabetic, wound healing, and abortifacient activities. Few studies on toxicity exhibited that most extracts from all parts of this plant did not show any signs of toxicity at higher doses according to histological analysis.

Summary: Phytochemical analysis of *H. rosa sinensis* documented that the main bioactive compounds responsible for its medicinal effects are namely flavonoids, tannins, terpenoids, saponins, and alkaloids. The in vitro antibacterial activity of *Hibiscus rosa-sinensis* flower extract against human pathogens was extensively seen. The Crude preparations of the different parts of *Hibiscus rosa-sinensis* have been traditionally used in folk medicine for various purposes. This review summarizes the phytochemical properties, pharmocology, medicinal uses and antibacterial activity of *Hibiscus rosa sinensis* against enteric pathogens.

KEYWORDS :

INTRODUCTION

China rose or "Queen of tropics" is often a popular name for the gorgeous flowering plant *Hibiscus rosa-sinensis*, as it is mainly found in south-east China and some islands in the Pacific and Indian Ocean. *Hibiscus* is one of Hawaii's admired national plants, and it is often seen worn in hair for cultural occasions (1). This plant belongs to the subkingdom *Magnoliophyta* and to the class *Magnoliopsida*, meaning that it is a vascular plant that produces seeds. It belongs to the family *Malvaceae*, and it is one of the 300 species of the genus *Hibiscus* (2). In addition, the juice extracted from the leaves and flowers has been used since a long time ago as natural remedy for some diseases and painful symptoms, as well as in herbal cosmetics as wilted (3). Dark flowers extract is used to make eyeliners, and in shoe-blacking (4). It was believed that the species was given the name "*rosasinensis* which means "Rose of China" in Latin, by the famous Swedish biologist, Carolus Linnaeus in the early 1750s (5).

Traditionally, *Hibiscus* flowers has been reported to possess antitumor properties, as well as have been used as analgesic, antipyretic, anti-asthmatic, and anti-inflammatory agents. Several studies have proved the presence of anti-oxidant, anti-fungal, and antimicrobial properties in flowers of *Hibiscus rosa-sinensis* (6). Research on extracts of stems, roots, leaves, and flowers from *Hibiscus* have revealed that its phytochemical components contributed to beneficial findings to human's health (7).

Current scientific literature suggests that more than 50% of today's clinical medications were of natural product origin. Many of them have played a significant role in pharmacological industry and in developing better therapies for various diseases (8). This plant is economically very essential owing to the herbal products and medicinal uses. Because of insuffiecient current pharmacological information, there is not much scientific research or clinical trials conducted on the chemical extracts of *Hibiscus rosa-sinensis* that could be crucial in exploring its fast potential medicinial applications (9).

Plants contain secondary metabolites, which are organic compounds that are not directly involved in the normal growth, development, or reproductions of organisms but often play an important role in plant defenses (10). Examples include alkaloids, glycosides, terpenoids, phenols, tannins, flavonoids and saponins. The importance of natural products in modern medicine has been discussed in recent reviews and research (11).

Hibiscus rosa-sinensis, a highly potential functional and valuable medicinal plant, has been reported in the ancient medicinal literature with beneficial effects in various disorders of humans. This is a tropical shrub, with large, glossy green leaves and spectacular trumpet shaped flowers. Its medicinal values have been mentioned in traditional folk medicines for variety of diseases. Recent studies shown that *Hibiscus rosa-sinensis* extract from different part of plant has significant protective effects (12), thus these plants have great medicinal potential for the therapy of infection. Flowers and leaves are found to possess antioxidant, antifungal, anti-infectious, antimicrobial, anti-inflammatory, anti-diarrheic and anti-pyretic activity (13).

Recent studies about the phytochemical constituent and antibacterial activity of methanolic extract of leaf and flower prove the antibacterial potential of *H. rosa-sinensis* extracts (14). Many chemical constituents have been isolated from this plant (15). Traditionally the flower can be use as antiasthmatic agent.

There is very wide range of pathogenic bacteria so there are many varieties of diseases caused by them. Medicinal plants have been using to cure many diseases from the ancient time. Plants are the best sources of natural products to maintain human health. Due to development of microbial resistance on antibiotics, researchers are trying to introduce antimicrobial activity of plants. Natural antimicrobial compounds in plants have been found to possess antimicrobial activity. Interest has been reviving in herbal medicine due to fewer side effects and limited ability of synthetic pharmaceutical products to control major diseases. Herbal treatment is one possible way to treat disease caused by multidrug resistant bacteria (16).

Hibiscus rosa-sinensis is very popular in India. It is an evergreen shrub which is in leaf all the years. Its beneficial

effects are also reported in ancient Indian Medicinal literature. *H.rosasinensis* flowers are used in the treatment of excessive and painful menstruation, venereal diseases, mumps, sores and coughs (17). Flower extracts of *H. rosasinensis* was tested against Gram Positive- *Staphylococcusaureus* and Gram Negative- *Salmonella typhimurium*, *E.coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*.

Food poisoning is considered as one of the most common cause of illness and dealth in developing countries (18). Most of food poisoning reports are associated with bacterial contamination especially members of Gram negative bacteria like *Salmonella typhi*, *Escherichia coli* and *Pseudomonas aeruginosa* (19). Other Gram positive bacteria including *Staphylococcus aureus* and *Bacillus cereus* have been also identified as the causal agents of food borne diseases or food spoilage (20). Prevention of food spoilage and their etiological agent is traditionally achieved by the use of chemical preservatives (21). Despite of the proven efficiency of these chemical preservative in prevention and outbreak control of food poisoning diseases, their repeated applications has resulted in the accumulation of chemical residues in food and feed chain, acquisition of microbial resistance to the applied chemicals and unpleasant side effects of these chemicals on human health (22).

Because of such, efforts have been focused on developing a potentially effective, healthy safer and natural food preservative. Within these contexts is the utilization of plant extracts as anti microbial agents for food preservation (23). These plant extracts considered as natural sources of antimicrobial agents, regarded as nutritionally safe and easily degradable (24). The antimicrobial activity exhibited by plant extracts against food poisoning bacteria has been demonstrated by several researchers (25). Moreover, antimicrobial activities of different natural substances such as medicinal plant extract have been investigated against food borne bacteria. Antibacterial activity of *Hibiscus rosa-sinensis* leaf and flower extract and its fractions showed a highly antimicrobial activity against Gram positive (*S. aureus* and *B. cereus*) and Gram negative bacteria (*E. coli* and *S. typhi*) causing food poisoning and these extracts can be used for prevention of food borne diseases or as preservative in food industry (26).

The range of pathogenic bacteria is wide and so is the variety of diseases caused by them. Despite the existence of potent antimicrobial agents, resistant or multi-resistant strains are continuously emerging, imposing the need for a continuous search and development of new drugs (27). Hence, many efforts have been exploited to discover new antimicrobial compounds from various kinds of sources such as soil, micro organisms, animals and plants. One such resource is medicinal plants, and their systematic screening may result in the discovery of novel effective compounds. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. There has been a revival of interest in herbal medicines due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases.

ANTIBACTERIAL ACTIVITY

In Vitro Antibacterial activity of *Hibiscus rosa-sinensis* flower extract against human pathogens.

Aqueous cold extractions of flower inhibited *B. subtillis, E. coli* with (17.00 ± 2.91), (14.50 ± 1.71) mm, respectively. Hot aqueous extraction showed an antibacterial activity against *E. coli*, *Salmonella* sp. at (11.66 ± 3.14), (10.60 ± 3.09) mm. The hot and cold extractions had very low inhibition effects against *B.subtillis, P. aeruginosa* at (1.00 ± 0.81), (0.00 ± 0.00) mm and *Staphylococcus sp., Salmonella sp.* (8.00 ± 1.63), (8.76 ± 2.71) mm.

Methanol solvent extraction showed a highest zone of inhibition recorded against *B. subtillis, E. coli* as (18.86 ± 0.18), (18.00 ± 1.63) mm followed by ethanol solvent extraction showed maximum zone of inhibition recorded against *Salmonella* sp. with (20.4 ± 1.54) mm.

The crude protein from flower shows a maximum inhibitory zone observed against *Salmonella* sp, *E. coli* viz., (16.55 ± 1.16) and (14.30 ± 2.86) mm. Pure protein showed zone of inhibition against *Staphylococcus sp, E. coli* such as (11.4 ± 1.74), (12.25 ± 0.97) mm (28).

Potential Antibacterial activity of *Hibiscus rosa-sinensis* Linn flowers extracts.

Methanol extract of *hibiscus* exhibits highest extraction yield value (29.75 g), followed by Ethanol, water and Ethyl acetate as (22.95, 22.25 and 20.90 g) respectively Furthermore, methanol has stronger extraction capacity which could be helpful in extracting greater number of active constituents responsible for antibacterial activity, this result in agreement with (Tiwari *et al*., 2015; Khan *et al*., 2014 and Abdelaleem *et al*., 2016).

The phytochemical analysis of *Hibiscus rosasinens* flower, the methanol extract of *Hibiscus rosasinens* flower contained varied type of phytochemical compound include Alkaloids, Glycosides, Carbohydrates, Phytosterols, Phenolic compound, Tannins, Saponins, Flavonoids, and Proteins and Aminoacids, and Alkaloids, Glycosides, Carbohydrates, Phytosterols, Phenolic compound, Tannins, and Flavonoids, in water extract, Alkaloids, Glycosides, Carbohydrates, Phytosterols, Phenolic compound, and Tannins in ethanol extract, Alkaloids, Glycosides, Carbohydrates, Phytosterols and Flavonoid in Ethyl acetate extract, this finding in agreement with study the phytochemical constituent and antibacterial potential of flower and leaf methanolic extract of H. rosasinensis and reported that, the phytochemical analysis showed present of alkaloid, glycoside, flavonoid, tannin and phenol in *H. rosasinensis* leaf extract, and alkaloid, protein, steroid, and carbohydrate in *H. rosasinensis* flower extract.

Antibacterial activity of methanol extracts prepared from flowers of *H. rosasinensis L.*, were screened against some bacteria such as *Escherichia coli, Streptococcus pyogenes* and *Staphylococcus aureus*. All flower methanol extracts at high concentration exhibited antibacterial potentiality of *H. Rosasinensis*.

In the present investigation flower methanol extract screened for antibacterial activity against *Escherichia coli*, *Streptococcus pyogenes* and *Staphylococcus aureus*. Antibacterial activity has been obtained in term of inhibition zone by cup- plate method, using three concentration of methanol flower extract. All bacteria represent increasing in inhibition zone diameter with increasing of methanol extract concentration. Among all treatment, highest zone inhibition observed from flower methanol extract at concentration 1mg/ml, for *E. coli* (27±0.12) mm, this finding agree with (28), followed by *Staphylococcus aureus* with (21±0.41) mm inhibition zone at the same flower extract concentration. Methanol extract at high concentration (1mg/ml) showed maximum inhibition zone against *E. coli*, *Staphylococcus aureus* and *Streptococcus pyogenes* as (27±0.12, 21±0.41, and 18±0.65) mm, followed by 0.5 mg/ml methanol extract showed maximum inhibition zone against *E. coli* as (17±0.21) mm, this finding in agree with the finding of (14), reported that the methanolic extract of *hibiscus* flower obtain antibacterial effect at high concentration (29).

Antibacterial activity of *Hibiscus rosa sinensis* and *Calendula offcinalis* flowers extract against various pathogens

Extract of *H. rosa-sinensis* shown zone of inhibition against

Staphylococcus aureus(23mm), *Salmonella typhimurium* (10mm), *E.coli*(11mm), *Proteus vulgaris*(17mm), *Pseudomonas aeruginosa*(20mm). *Staphylococcus aureus* showed maximum zone of inhibition. *Staphylococcus aureus* causes nosocomial infection, blood infection, pimple, boils and other skin infection, scarlet fever etc. It also acquired resistance for most of the antibiotics. Flower extract of *H. rosa-sinensis* also inhibit the growth of other disease causing bacteria. This indicate that flower extract have potential for treating infection caused by *Staphylococcus aureus* and other clinical isolets.

Our study concluded that plant extracts have antimicrobial potential. These flower patels contains flavonoids, cyanidine, querecetin, calcium oxalate, thiamine, ribofalavin, niacin, ascorbic acid and oxalic acid. Further biochemical and pharmacological investigation would be required for medicinal use (30).

Phytochemical Screening and Antibacterial activities of *Hibiscus Sabdariffa* Linn leaf extracts.

The result of phytochemical screening of methanolic extracts of *Hibiscus sabdariffa L* leaves revealing the presence of alkaloids, saponins, steroids, flavonoids and tannins. The distribution of saponins, flavonoids, tannins and steroids in the extract confirmed its use as an antimicrobial agent. The antibacterial activity of methanolic extract of *Hibiscus sabdariffa* L. against *Salmonella typhi, Staphylococcus aureus* and *Escherichia coli*. The activity against all the test organisms occurred at all concentrations with highest inhibition zone of 18mm at 100mg of *Escherichia coli* which had the highest zone of inhibition (31).

In this study of Antibacterial activity of the ethanol extracts of *Hibiscus rosa-sinensis* leaves and flowers against clinical isolates of bacteria.

The extracts of *H. rosa-sinensis* flowers showed stronger antibacterial activity than that of leaves. The maximum zone of inhibition (29 mm) was observed against *S. aureus*, followed by *P. vulgaris* (25 mm), *P. aeruginosa* (24 mm) and *Citrobacter* sp. (24 mm) and the lowest against *S. typhimurium* (13 mm) at the highest amount of flower extracts (100 mg/well). All the test bacteria responded to the extracts in a dose-dependent manner. However, K. *pneumoniae* was found to be resistant to the flower extracts at any of the applied doses (50 and 100 mg/well). Thus the extracts of *H. rosa-sinensis* flowers showed strong antibacterial activity against *S. aureus* and moderate activity against *P. vulgaris, P. aeruginosa* and *Citrobacter sp*.

S. aureus was also the most sensitive bacterium to the extracts of *H. rosa-sinensis* leaves. This was followed by *S. typhimurium* and *E. coli*. Rest bacterial isolates were found to be resistant to either of the applied doses of the extracts of leaves.

In the bacteriological enumeration method, all the bacteria showed significant percentage of sensitivity to the plant extracts. *S. typhimurium*, *E. coli* and *P. aeruginosa* showed 100 % sensitivity to both the extracts of leaves and flowers at the applied dose of 100 mg/ml of nutrient broth. Some of the bacterial isolates that showed resistance in agar-well diffusion method were found sensitive in bacteriological enumeration method. This might be due to the inferior diffusion of extracts in the agar-well diffusion method.

S. aureus is a leading cause of community-acquired and hospital-acquired infections. It is commonly found on the skin and in the nose of healthy people. Occasionally, *staphylococci* cause infections that are minor, such as pimples, boils, and other skin conditions or sometimes serious and fatal, such as blood infections, carditis, meningitis, or pneumonia. It produces numerous toxins including super-antigens that cause unique disease entities such as toxic-shock syndrome and staphylococcal scarlet fever, and has acquired resistance to practically all antibiotics. Over the past 50 years, *S. aureus* has undergone incremental changes in genetic complement that have resulted in the emergence of antibiotic-resistant strains and appear to be successful in transmitting and causing disease in the hospital setting. In the study, *S. aureus* showed sensitivity to the extracts prepared from both leaves and flowers. This indicates that the extracts of leaves and flowers have immense potential to be used in treating infections caused by *S. aureus* (32).

Antioxidant and antibacterial activities of *Hibiscus* and *Cassia* flower extracts.

The antibacterial activity of aqueous and ethanolic extracts of *Hibiscus* flowers against various Gram-positive and Gram-negative pathogenic bacteria. Results showed aqueous and ethanolic extracts of *Hibiscus* flowers (at the concentration of 100 and 50 mg/mL) to selectively inhibit the growth of *S. typhimurium* and *S.aureus* respectively. However, none of the tested sample extracts showed inhibitory effects against *B. subtilis*, *E. coli*, *L. monocytogenes* and *S. enteritidis*. Overall, the zone of inhibition ranged from 9–14 mm for *hibiscus* extracts, respectively. Reports available have shown crude plant extracts to exhibit higher antibacterial activities against Gram-positive Bacteria than Gram-negative bacteria. This has been attributed to structural variations observed in the bacterial cell envelope (including those of cytoplasmic membrane and cell wall components) between Gram-positive and Gram-negative bacteria (33).

A survey on *Hibiscus rosa-sinensis*, *Alcea rosea* L. and *Malva neglecta* Wallr as antibacterial agents.

The highest zone of inhibition followed by the ethanolic extract of *Hibiscus rosa-sinensis* against *S. epidermidis* and *S.aureus* (20mm). On the other hand, *B. cereus, S. typhi* and *K. pneumoniae* were resistant to *H. rosa* extracts [except leaf extracts of *H. rosa* at 0.4g/mL]. We found that the extracts of *H. rosa-sinensis* flowers showed stronger antibacterial activity than that of leaves (34).

An update review on *Hibiscus rosa sinensis* phytochemistry and medicinal uses.

The methanol extracts prepared from the leaves of the *H. rosa-sinensis* were shown to have antimicrobial activities against *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter aerogenes*, and *Streptococcus pyogenes*. The maximum zone of inhibition was observed at 13 ± 00 mm and it was against *E. coli* followed by 12 ± 00 mm against both *S. aureus* and *E. aerogenes* at 80 μg/ml concentration of leaves methanolic extract.

In another study conducted using disc diffusion method, **aqeuous leaves** extracts of 40 mg/ml showed maximum zone of inhibition against *Bacillus subtilis* (14.00 ± 1.05 mm), *E. coli* (12.30 ± 0.95 mm) and *S. aureus* (11.00 ± 1.20 mm), while the **methanol** extract showed the following zones of inhibitions against *B. subtilis* (18.82 ± 0.18 mm), *E. coli* (17.30 ± 0.51 mm), *S. aureus* (15.20 ± 0.90 mm).

Antibacterial activity has been also observed using disc diffusion method against *E.coli* and *S. aureus* at different concentrations of **methanolic flower** and **leave** extracts varying from 31.25 to 500 mg/ml. These were compared with positive control gentamicin (1 mg/ml) and methanol as negative control. In both extracts types, the antibacterial activity was increased with extract concentration. The highest zones of inhibitions observed were at concentration 500 mg for *E. coli* were 23 ± 1.01 mm and 13.75 ± 0.99 mm for leaf and flower methanolic extracts respectively. Conversely, for *S.aureus*, methanolic leaf and flower extracts gave 19.33 ± 0.29 mm and 9.75 ± 0.76 mm as highest zones of inhibitions

respectively at concentration 500 mg. In this study, carbohydrates, phytosterols, and proteins were identified from flower extracts and glycosides, tannin, phenols, and flavonoids from leave extracts. However, alkaloids and saponins were found in both extracts.

The **cold aqueous** extractions of flowers gave highest zones of inhibition against *B. subtillis* as 17.00 ± 2.94 mm, and against *E. coli* as 14.50 ± 1.71 mm. In contrast, the **hot aqueous** extraction inhibited *E. coli* growth for 11.60 ± 3.14 mm, and *Salmonella* sp. for 10.66 ± 3.09 mm as maximum zones of inhibition. The methanol extracts showed highest zones of inhibition against *B. subtillis* as 18.86 ± 0.18 mm, against *E. coli* as 18.00 ± 1.63 mm. The ethanol extracts gave zones of inhibition against *Salmonella* sp. as 20.40 ± 1.54 mm, and *P. aeruginosa* as 16.30 ± 0.94 mm. All these mentioned microorganisms are considered as human pathogens. The antibacterial activities of both **pure and crude protiens** from flowers were also investigated such that crude protein inhibited the growth of *Salmonella* sp. for 16.55 ± 1.16 mm, and *E. coli* for 14.30 ± 2.86 mm as maximum inhibition zones, compared to pure protein which inhibited *Staphylococcus* sp for 11.4 ± 1.74 mm, *E. coli* for 12.25 ± 0.97 mm. Moreover, the pure and crude *H. rosa-sinensis* flower proteins were run in poly acrylmide gel electrophoresis (PAGE), which resulted in various bands for the crude protein sample. This suggests that the crude extract may contain components such as flavonoids, triterpenoids, tannins, alkaloids. For example, flavonoids are considered antimicrobial agent as they are able to complex with components of the bacterial cell wall and eventually deteriorate it.

The antibacterial activity of **root extracts** from *H. rosa sinensis* was also investigated. It was reported that using disc diffusion method, the ethyl alcohol root extracts inhibited the growth of *S. aureus* for 2 cm and *E.coli* and *B. subtilis* for 1.5 cm as highest zones of inhibition at the concentration 7.5 μg/ml. Similarly, methanol flower extracts exhibited highest inhibition zones at the concentration of 1 mg /ml against *E.coli* (27 ± 0.12 mm), *S. aureus* (21 ± 0.41 mm), and *Streptococcus pyogenes* (18 ± 0.65 mm), compared to 10μg of Chroramphenicol which was used as a positive control and resulted in a zone of inhibition of approximately 24 mm. Compared to other extracts such as ethyl acetate extract, ethanol extract, and water extract, all phtochemicals that were involved in the antimicrobial activity were detected from the methanol extract (9).

CONCLUSION

Flavonoids, tannins, terpenoids, saponins, and alkaloids are the main phytochemicals as they are present in different extracts, and are more likely responsible for their biological activities. Lower toxicity of this plant can be an advantage to qualify it to be used as new therapeutic agent.

This review has presented a comprehensive summary of recent studies on the phytochemistry and the medicinal uses of *H. rosa sinensis*.

Therefore, the flowers, leaves, and roots extracts can be used as vital antibiotics components to treat diseases caused by the different strains of bacteria. Even these results are encouraging, precise assessment is absolutely necessary before undergoing further pharmacological evaluation. Because pathogenic microorganisms are getting resistant to current antimicrobial agents, scientific research has continued to search for other sources of antimicrobial compounds.

Moreover, clinical trials on the toxicity of this plant and its pharmocological effects must be carried out to assess its safe application and precede no side effects.

From the above study, it is concluded that cold and hot aqueous, methanolic extracts, crude extraction of flowers and methanolic extract of flowers and leaves of *Hibiscus rosa-sinensis* shows the maximum zone of inhibition against Gram negative bacteria *Escherichia coli*. Ethanolic extracts of flowers and leaves of *Hibiscus rosa-sinensis* show the maximum zone of inhibition against Gram positive bacteria *Staphylococcus aureus* whereas both are enteric pathogens.

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