Studies on bioactive compounds and antimicrobial potential of Hibiscus Plant

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Abstract- The aim of the present study was to investigate the presence of biologically active compounds (phyto-chemicals) and antimicrobial potential in leaves and flower extracts (aqueous, ethanol and acetone) of *Hibiscus* plant against pathogenic bacterial and fungal strains. Ethanol and acetone extract shown good number of bioactive compounds compared to aqueous extracts. All the tested biological compounds like protein and amino acids, carbohydrates, proteins, glycosides, tannin, phenol, alkaloids, terpenoids, flavonoids and saponin were abundantly present in plant extracts. Antimicrobial activity of different extracts of Hibiscus rosa-sinensis were tested against bacterial strains (*Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa)* and fungal strains (*Aspergillus niger, Aspergillus flavus, Penicillium crysogenum)* Antimicrobial activity was carried out by agar disc diffusion method. The result showed that, all extract showed antifungal activity against all isolated fungal strains. The acetone extracts of flower exhibited maximum inhibitory activity against the *P.crysogenum, A. niger and A. flavus*. While the ethanol extract of leaf showed minimum inhibition zone against bacterial strains. The acetone extract gave better antimicrobial activity in comparison to other extracts. Our findings prove that, the flower of hibiscus plants have medicinal antibacterial activities and can use against bacteria.

Keywords: *Hibiscus,* Antimicrobial, Bioactive compounds.

Introduction

Plants have evolved the ability to synthesize chemical compounds that help them to defend against attack from a wide variety of predators such as insects, fungi and herbivorous mammals. By chance some of these compounds whilst being toxic to plant predators turn out to have beneficial effects when used to treat human diseases. (Reena Patel *et al*, 2012). Importance of the plants basically originates due to the presence of specific biological active classes of organic compounds (Shivananda,2007). Many of the herbs and spices used by humans are useful medicinal compounds (Uddin, 2010) More than 80% world's population still emphasize to use traditional and old medicinal system such as Homeopathy, Unani, Ayurveda, Sidha, Naturopathy etc. *Hibiscus rosa-sinensis is* (hibiscus plant) is member of the family Malvaceae, grow as green herbaceous plant in tropical

regions. It 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. Flowers and leaves are found to possess antioxidant, antifungal, anti-infectious, antimicrobial, anti-inflammatory, anti-diarrheic and antipyretic activity (David and Leonard, 1998). Traditionally the flower can be using as anti-asthmatic agent (Zhao et al., 2010). The aim of this study is to evaluate the anti-microbial efficacy of the plant extract of *Hibiscus rosa-sinensis is* against *Penicillium crysogenum, Aspergillus niger, Aspergillus flavus, Staphylococcus aureus, Bacillus subtilis and Pseudomonas aeruginosa. And* investigated the presence of biologically active compounds (phytochemicals) in extracts.

Materials and methods

Collection of plant material: The leaves and flowers of. *hibiscus* were collected from the Botanical Garden, Department of Botany, D.A.V. (PG) College, Muzaffar-nagar, UP, India. The different plant parts were washed with running tap water and subjected to crude extract preparation at room temperature for further studies.

Preparation of crude extract: The leaves and flowers of the plant *hibiscus* were used for crude extract preparations separately for phytochemical and antimicrobial analysis. 10 g (fresh weight) of different parts (leaf and flower) of *hibiscus* were homogenized in 3-5 folds of aqueous and organic solvent (50%, ethanol and acetone) in pestle and mortar at room temperature. The extracts were filtered through sterilized Whatman filter paper and filtrate was centrifuged at 10000 rpm at 4^0C for 5 minutes. The clear supernatant was used as crude extract for phytochemical analysis and antimicrobial testing. Extracts were kept at 4 °C for the further analysis.

Isolation, purification and identification of microbial strains from soil and spoiled fruits: The microbial strains were isolated from soil by serial dilution method. (Cappuccino and Sherman, 2005; Aneja , 2009) and by some spoiled fruits. The isolated colonies of *Penicillium crysogenum, Aspergillus niger and Aspergillus flavus* were streaked onto the fresh SDA plates while *E. coli, Bacillus sp. and Pseudomonas sp*. were streaked onto the fresh NAM plates.

Screening of antimicrobial activity: Antimicrobial activity of plant extract was screened by agar disc diffusion method on SDA plate for fungal strains and NAM plates for bacterial strains and measure the zone of inhibition in mm. (Khokra *et al*, 2008).

Phytochemical screening of different parts of *T. corymbosa*: Phytochemical tests were carried out in the aqueous and organic extract of *T. corymbosa* using standard methods to identify the phytochemical constituents as described by Sofowara (1993), Trease and Evans (1989), Omoya and Akharaiyi (2012), Jyothiprabha and Venkatachalam (2016), Harborne and Williams (2000).

Screening for Tannins: 5 ml each of the extracts were stirred separately with 100 ml distilled water and filtered. One ml ferric chloride reagent was added to the filtrate. A blue-black or blue green precipitate was an indication of the presence of tannins.

Screening for Terpenoids: 5 ml of extract was taken in a test tube and 2 ml of chloroform was added to it followed by the addition of 3 ml of concentrated sulphuric acid. Formation of reddish-brown layer at the junction of two solutions confirms the presence of terpenoids.

Screening for Flavonoids: A pinch of zinc dust was added to 2 ml of extract followed by the addition of 1 ml concentrated HCl. Appearance of pink colour indicate the presence of flavonoids

Screening for Saponins: 5 ml each of the extracts were mixed with distilled water and shaken separately in a test tube. Frothing, which persists on warm heating was taken as preliminary evidence for the presence of the saponins.

Screening for Glycosides: 5 ml extract was mixed thoroughly with 1 ml of glacial acetic acid and 1 ml of 5% FeCl3 solution in a test tube. 1ml of concentrated sulphuric acid was added to the above reaction mixture carefully along the side of test tube. Development of green-blue colouration shows the presence of glycosides. (Kellar- Kiliani test).

Screening for Alkaloids: Mayer's test: 1 ml of every extract mix with a drop of Mayer's chemical agent is additional by the aspect of the test tube. A creamy or white precipitate indicates the presence of alkaloids.

Screening of Phenols: Few drops of 10% lead acetate solution were added to 5ml of test solution. Formation of white precipitates indicates the presence of phenol in the test solution.

Results and Discussion

In the present investigation bioactive substances (phytochemical) analysis has been performed in different extracts of hibiscus plant (leaves and flower) which showed the presence of various phytochemical constituents, glycosides, tannin, phenol, alkaloids, flavonoids, terpenoids, phenols and saponin (as shown in Table 1). All the phytochemicals are abundantly found in flower extract (aqueous, ethanol, acetone) followed by in leaf extract respectively. This phytochemical screening is more prominent in acetone extract and ethanol extract as compared to aqueous extract as bioactive compound are organic in nature Presence of high level of total phenols, flavonols, flavonoids, and anthocyanins has been reported in different flowers and their extracts (Cai et al., 2004; Gouveia et al., 2013; Yanget al., 2012; Wijekoon et al., 2011) and soluble in organic solvent. Presence of high level of total phenols, flavonols, flavonoids, and anthocyanins has been reported in different flowers and their extracts (Cai et al., 2004; Gouveia et al., 2013; Yanget al., 2012; Wijekoon et al., 2011)

The presence of phytochemicals as such; flavonoid, alkaloid, tannin showed cytotoxic effect (Chowdhury et al. 2017). Additionally, cholesterol-lowering, as well as cytotoxic qualities, anti-bacterial, anti-viral properties, are credited to the presence of saponin (Bailly & Vergoten, 2020). The Flavonoids and phenols are major compounds that act as antioxidants or free radical scavengers (Bhandary *et al.*, 2012). Tannin shows an anticancer property that is perceptible from its inhibitory activity towards growth while the phenolic compound, tannin, terpenoid,

flavonoids possess an ant-helmintic property so the plant Zanthoxylum, Acorus could be used to treat stomach problems (Nath & Yadav, 2016).

Table 1: Screening of bioactive compounds (phytochemicals) in leaf and flower of *Hibiscus rosa-sinensis is* in aqueous, ethanol and acetone extracts.

S.No.	Name of bioactive compound	Leaf			Flower		
		Aqueous	Ethanol	Acetone	Aqueous	Ethanol	Acetone
1	Alkaloids	+++	+	+++	+++	+++	+++
2	Glycosides	++	++	+++	+++	+++	+++
3	Tannin	+	++	+++	+++	+++	+++
4	Terpenoids	++	+++	++	+	++	+++
5	Phenols	++	+++	+++	+	++	+++
6	Saponin	+++	+++	+++	+++	+++	+++
7	Flavonoids	++	++	++	+++	+++	+++

+ = presence, - = absence, ++= moderate, +++= abundant

Table 2 showing the antimicrobial potential of *Hibiscus rosa-sinensis is* leaves and flower in aqueous, ethanol and acetone extract against isolated fungal and bacterial strains. It is observed that the flower extract prepared in acetone showed the maximum inhibition zone against isolated bacterial strains i.e. *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa and fungal strains (Aspergillus niger, Aspergillus flavus, Penicillium crysogenum*). While the extract of leaf in distilled water, acetone and ethanol showed less activity against fungal strain *Aspergillus niger*, *Aspergillus flavus* and *P. crysogenum*. The minimum inhibition zone is found in distilled water leaf extract against *A. niger* while the maximum zone found against *Pseudomonas aeruginosa* in flower acetone extract. This finding in agree with the finding of (Uddin et al., 2010), reported that the methanolic extract of Hibiscus flower obtain antibacterial effect at high concentration. Polyphenols, flavonoids and tannins present in a sample might be responsible for the observed antibacterial activity. These compounds are generally produced by plants as a mode of defense against microbial infections. (Scalbert, 1991)

Table-2 Antimicrobial screening in leaf and flower of *Hibiscus rosa-sinensis is* in aqueous, ethanol and acetone extracts

S.No.	Name of Microorganism	Leaf extracts of hibiscus (Inhibition zone in mm± SD)			Flower extracts of hibiscus (Inhibition zone in mm± SD)		
		Aqueous	Ethanol	Acetone	Aqueous	Ethanol	Acetone
1	***Penicillium crysogenum***	12.2±0.21	15.1±0.25	19.3±0.22	15.5±0.21	18.4±0.31	22.2±0.31
2	***Aspergillus niger***	11.1±0.18	18.2±0.26	12.4±0.22	11.6±0.31	15.7±0.23	19.4±0.23
3	***Aspergillus flavus***	14.4±0.27	14.1±0.25	14.2±0.31	14.7±0.25	20.1±0.26	24.8±0.26
4	***Pseudomonas aeruginosa***	16.8±0.35	19.6±0.33	21.7±0.19	19.9±0.22	24.8±0.29	29.7±0.29
5	***Staphylococcus aureus***	17.3±0.35	23.5±0.24	20.1±0.23	20.2±0.21	28.4±0.34	27.5±0.25
6	***Bacillus subtilis***	18.4±0.29	21.2±0.25	22.7±0.22	18.4±0.32	25.5±0.12	28.6±0.22

Conclusions

From the study, it could be concluded that plants are a great source of phytochemicals that could be utilized in curing various ailments. The bio active substances in plants are produced as secondary metabolite i.e. Tannin, terpenoids, flavonoid, saponin, alkaloids, glycosides, phenols. This phytochemical screening test may be helpful in the screening of bioactive compound and eventually may provide a therapeutic platform to develop new drugs. The flower acetone extract of *Hibiscus* plant can be considered to be as equally potent as the most of effective artificial antibiotics.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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