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RESEARCH ARTICLE

Comparative study of phytochemical antibacterial activity, antifungal and antioxidant activity *Hibiscus cannabinus* using various solvents

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Abstract

Kenaf, *Hibiscus cannabinus*, is a plant in the Malvaceae family. *Hibiscus cannabinus* belongs to the genus Hibiscus and is probably native to Southern Asia, In Ayurvedic medicine, the leaves are used in the treatment of dysentry and bilious, blood and throat disorders. The powdered leaves are applied to Guinea worms in Africa. The *Hibiscus cannabinus* leaves extract of four solvents such as hexane, chloroform, ethyl acetate, methanol were used in the present study. The soxhlet extractor was employed in this study. The plants were screened for the presence of phytochemicals and their effect on 2,2-Diphenyl-1-picryl-hydrazyl radical (DPPH) was used to determine their free radical scavenging activity. Phytochemical screening of the plant showed the presence of glycosides, steroids, falvonoids, terpenoids and tannins except alkaloids. Concentration of the plant extract required for 50% inhibition of DPPH radical scavenging effect was recorded as 0.343 mg/ml, 0.571 mg/ml, 0.325 mg/ml, 0.036 mg/ml for hexane, chloroform, ethyl acetate and methanol. The antimicrobial activity of *Hibiscus cannabinus* leaves shows a positive result in the sample by which it is extracted using chloroform as a solvent.

INTRODUCTION

Treatment by chemotherapy of infectious diseases has proved to be a continuous struggle and for many years, the control of various diseases was confined to symptomatic cure which included personal hygiene, isolation of patient and good health. Chemotherapy started with the vaccination discovered by Edward Jenner but the term 'chemotherapy' was originally coined by Paul Ehrlich who discovered the first effective chemotherapeutic agent-arsphenamine/salvarsan, which opened the door to future developments in chemotherapy and antibiotics.

The 20th century has witnessed a complete transformation in the treatment of infectious diseases. The antibiotic era began with therapeutic applications of sulfonamides in 1930's, followed by a "golden" period (1935-1970) with a flurry of discoveries of effective antibiotics. Success story of antimicrobial drugs decreased the mortality rate and generated a misconception in the late 1960's and early 1970's that infectious diseases had been conquered. However, 1980's saw a decline in the discovery of new agents for clinical use. This period with a reduced rate of introduction of new agents was accompanied by an alarming increase in drug-resistant microorganisms.

Antimicrobial drug resistance may occur due to a pre-existing factor in the microorganisms or it may be due to some acquired factors by genetic changes or non-genetic mechanisms. Antimicrobial resistance can be categorized as microbial, clinical, cross and parallel or co-resistance (Shah, 2005). The most common mechanisms underlying bacterial resistance are: Production of enzymes capable of inactivating the antimicrobials, altered binding sites,

altered membrane permeability and enhanced efflux of antimicrobials (Lode, 2008).

Enzyme production (β-lactamase) is the major mechanism of resistance to β-lactam antibiotics in many organisms while alterations in penicillin-binding proteins and decreased permeability of the bacterial outer membrane to β-lactams are the other rare mechanisms of resistance. Multiple drug-resistant microorganisms have increased around the world (Gould, 2009) and pathogens such as methicillin-resistant Staphylococcus aureus (MRSA).vancomycin-resistant Staphylococcus aureus(VRSA); vancomycin-resistant Enterococci (VRE); extended spectrum β-lactamases (ESBLs) producing- Escherichia coli and Klebsiella pneumoniae; penicillin resistant Streptococcus pneumonia; and Pseudomonas aeruginosa are becoming resistant to almost every available antibiotic drug (Zhang et al., 2006; Livermore, 2009).

The emergence and spread of antimicrobial resistance is an array of problems caused by various interconnected factors, many of which are related to over and misuse of antibiotics (Overbye and Barrett, 2005). Thus, antimicrobial resistance in addition to cost, toxicity and various side effects related to antibiotic consumption remains one of the biggest challenges all over the globe (DiazGranados et.al., 2008).This demands the development of new antimicrobial compounds to answer the problem posed by resistant and other microorganisms (Chopra et.al., 1997).

Medicinal plants are relied upon by 80% of the world's population and a major part of the traditional therapies involve the use of plant extracts or their active constituents (Newman and Cragg, 2007). Herbal medicine constitutes an integral part of all the major healthcare systems viz. Ayurveda, Siddha, Unani, Yoga, Homeopathy and Naturopathy. Among these systems, Ayurveda is the most developed and widely practised in Indian traditional medicinal system and a number of modern drugs owe their origin to these herbs which are used as homemade remedies to treat various ailments (Vaidya and Devasagayam, 2007).

Furthermore, the simultaneous determination of the compounds which are possibly responsible for any biological activity would aid in validating the folklore use of traditional plants in curing various microbial pathogenesis. Besides the availability of reports on antimicrobial activities of some indigenous medicinal plants, the medicinal flora of our country still remains virtually unexplore.

2. MATERIALS AND METHODS

2.1 Collection and identification of plant materials

Fresh leaves of *Hibiscus cannabinus* were collected from the local garden at Thiruvallur, Tamil Nadu.

2.2 Extraction of plant materials

The leaves of *Hibiscus cannabinus* were air dried in the shadow for 24 hrs, after which it was ground into an uniform powder. The extracts were prepared by using various solvents such as hexane, chloroform, ethyl acetate, methanol. This dried powdered plant material was extracted by using soxhlet extractor.

3. QUALITATIVE ANALYSIS OF PHYTOCHEMICAL SCREENING

Phytochemical tests were carried out using methanolic extracts as follows:

3.1 Test for glycosides- Liebermann's test

2ml of sample was dissolved in 2ml of chloroform and then 2ml of acetic acid was added into it. The solution was cooled in ice. Sulphuric acid was added carefully. A colour change from violet to blue to green indicates the presence of a steroidal nucleus.

3.2 Tests for Steroids

A red colour in the lower chloroform layer when 2ml of organic extract was dissolved in 2ml of chloroform and 2ml concentrated sulphuric acid was added in it, indicates the presence of steroids.

3.1.1. Total phenol estimation

A mixture was prepared dissolving 0.1mg of sample in 1ml of methanol. From the mixture, 20µl was taken and 180µl water is added. Then 0.5ml of folin phenol reagent 0.5ml of water 1ml of 7.5% sodium carbonate was added to the mixture. Then, it was kept 2hrs under incubation and the absorbance was read at 726nm by spectrophotometer. Gallic acid was used as phenol standard and expressed as gallic acid equivalent.

3.1.2. Phytochemical analysis

The phytochemical analyses of each sample were performed for alkaloids, tannins, saponins, flavonoids

and terpenoids.

3.3 Test for alkaloids

Some small amount solvent free extract was dissolved in dilute Hcl. 1.2ml of this extract was mixed with 0.1ml of Mayer's reagent.

3.4 Test for tannins

Small amount of extract was dissolved in 2ml of distilled water and it was mixed with a few drops of 1% ferric chloride.

3.5 Test for saponins

A pinch of extract was dissolved in 1ml of distilled water. It was warmed in the heating mantle for 2 minutes at 60. Then 0.5ml of distilled water was added to it and shaken well.

3.6 Test for falvonoids

A pinch of the extract was dissolved in 5ml of distilled water. 10% of sodium hydroxide was prepared and mixed with the extract. The yellow colour disappears by the addition of dilute Hcl.

3.7 Test for terpenoids

A small amount of extract was dissolved in 1ml of chloroform and 1ml of conc. Sulphuric acid.

4. Free radical scavenging ability by the use of a stable DPPH radical (1,1-diphenyl-2-picrilhydrazyl)

The effect of given samples on DPPH radical was estimated according to the procedure described by Von Gadow *et.al.* (1997). Two ml of 6 $\times 10^{-5}$ M methanolic solution of DPPH were added to 50 µl of a methanolic solution (10 mg ml^{-1}) of the sample. Absorbance measurements commenced immediately. The decrease of absorbance at 515 nm was continuously recorded in a spectrophotometer for 16 min at room temperature. Methanolic solutions of pure compounds (quercetin) were tested at 1 mg/ml concentration. The scavenging effect (decrease of absorbance at 515 nm) was plotted against the time and the percentage of DPPH radical scavenging ability of the sample was calculated from the absorbance value at the end of 16 min duration as follows

All determinations were performed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula of Yen and Duh (1994).

IP = $[(A_{C(0)} - A_{A(t)} / A_{C(0)})] \times 100$ Where $A_{C(0)}$ is the absorbance of the control at t = 0 min; and $A_{A(t)}$ is the absorbance of the antioxidants at t = 16 min.

4.1 *In vitro* antimicrobial activity

The antibacterial activity was determined by well diffusion methods (Holder and Boyce 1994). About 25 ml of molten Mueller Hinton agar was poured into a sterile petri plate (Himedia, Mumbai, India). The plates were allowed to solidify (OD adjusted to 0.6). 100 µl of pathogenic bacteria cultures such as *Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli* and *Pseudomonas aeruginosa* and one yeast *Candida albicans* were transferred onto plates individually and a culture lawn by using sterile L-rod spreader. After five min setting of the pathogenic bacteria, a sterile cork borer was used to make 5 mm well on the agar. The test samples were dissolved in methanol and loaded into wells with various concentrations such as 50 g/well, 100 g/well, 150 g/well and 200 g/well. The streptomycin added well served as positive control for bacteria and Clotrimazole served for fungi. The plates were incubated at 37°C in a 40 W florescent light source (~ 400 nm) for 24 h. The antibacterial activity was determined by measuring the diameter of the zone of inhibition around the well using antibiotic zone scale (Himedia, India).

5. RESULTS

Phytochemical screening of plant materials

The phytochemical screening of the *Hibiscus cannabinus* studies showed the presence of flavonoids, terpenoids, saponins, glycosides, steroids, tannins (Table 1), by four solvents. The *Hibiscus cannabinus* showed the absence of alkaloids in all solvents (Table 1).

Radical scavenging (antioxidant) activity

0.023, 0.056, 0.042, and 0.026mg/ml were recorded for Methanol in *Hibiscus cannabinus* respectively.

Fig 5.1 *Staphylococcus aureus*

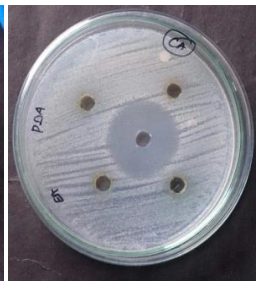

Fig 5.2 *Candida albicans*

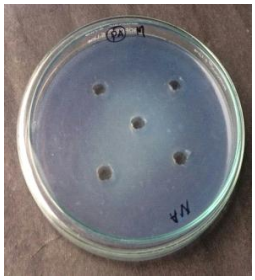

Fig 5.3 *Pseudomonas aeruginosa*

Fig 5.4 *Klebsiella pneumonia*

Table 5.2: Phytochemical screening of Hexane, Chloroform, Ethyl acetate, Methanol

Test	Hexane	Chloroform	Ethyl acetate	Methanol
Glycosides	_	_	_	+
Steroids	+	+	+	+
Alkaloids	_	_	_	_
Tannins	_	_	_	+
Saponins	_	_	_	+
Flavonoids	_	_	+	+
Terpenoids	_	+	+	+

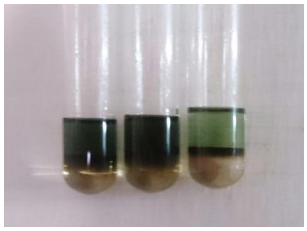

Fig 5.6 Presence of terpenoids

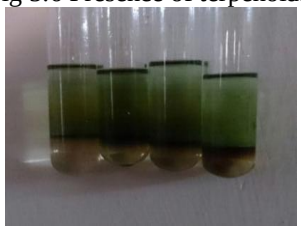

Fig 5.7 Presence of steroids

Fig 5.8 Phenol Estimation

Table 5.3: Total phenol estimation

Solvent	Spectrophotometer reading at 517 nm (µg GAE)	Result (mg GAE/g dry extract)
Hexane	0.303	490
Chloroform	0.463	756.66
Ethyl acetate	0.341	553.33
Methanol	0.454	741.66

6. DISCUSSION

Phytochemical screening of the plant revealed some differences in the constituents of the four solvents tested. Steroids, glycosides, saponins, falvonoids, terpenoids and tannins were tested positive for all solvents; alkaloids showed the absence of the all solvents. The plants exhibited potent antioxidant activity. The presence of flavonoids and tannins in all the plants is likely to be responsible for the free radical scavenging effects observed. Flavonoids and tannins are phenolic compounds and plant phenolics are

a major group of compounds that act as primary antioxidants or free radical scavengers14. The DPPH test provides information on the reactivity of the test compounds with a stable free radical. DPPH gives a strong absorption band at 517 nm in the visible region. When the odd electron is paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution is decolourised as the colour changes from deep violet to light yellow. The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract. The crude extract of Hexane appeared with a maximum inhibition of 44.67% at 0.343mg/ml. The Chloroform appeared (very least value) with a maximum inhibition of 7.85% at 0.571 mg/ml, followed by ethyl acetate which have less potency with a maximum inhibition of 47.53% at 0.325mg/ml. Methanol showed maximum inhibition of 94.06% at 0.036mg/ml. This study suggests that these plants possess antioxidant activities which can counteract the oxidative damage induced by the malaria parasite. This may be one of their mode of action in malarial therapy.

7. CONCLUSION

Extracts from *Hibiscus cannabinus* showed varying antioxidant (free radical scavenging) and antibacterial activities by using various solvents. The phytochemical analysis test of *Hibiscus cannabinus* showed the best result in methanol extract. The antimicrobial activity showed the best result in chloroform. The results suggest that the antioxidant activity is better in methanol solvent by using *Hibiscus cannabinus* plant.

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