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Extraction and Evaluation of Anti-oxidant Activity of *Hibiscus Cannabis. L*

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Research Article

ABSTRACT

According to the literature review, it was found that various activities was done and reported on Hibiscus cannabis L., But anthelminthic activity of the leaves of the plant Hibiscus Cannabis L were not reported. So, my present work is aimed to carry out the Extraction & Isolation, Phytochemical screening, and estimation of anti-helminthic activity, anti-oxidant activity of hydro alcoholic extract, ethanolic extract, and water extract of Hibiscus Cannabis L.

Introduction

A natural product [1-4] is a chemical compound or substance produced by a living organism - found in nature that usually has a pharmacological or biological activity for use in pharmaceutical drug discovery and drug design. A natural product can be considered as such even if it can be prepared by total synthesis.

Natural products as medicines: History and the earliest known medicines to man

For thousands of year's natural products have played a very important role in health care and prevention of diseases [5-8]. The ancient civilizations of the Chinese, Indians and North Africans provide written evidence for the use of natural sources for curing various diseases.

According to recent studies conducted by the World Health Organization (WHO), about 80% of the world's population relies on traditional medicine [9-25] About 121 drugs prescribed in USA today come from natural sources, 90 of which come either directly or indirectly from plant sources.4 Forty-seven percent of the anticancer drugs in the market come from natural products or natural product mimics [26-30].

Types of Natural products

Natural products from microorganisms

Microorganisms as a source of potential drug candidates were not explored until the discovery of penicillin in 1929 [31-45]. Since then, a large number of terrestrial and marine microorganisms have been screened for drug discovery. Microorganisms have a wide variety of potentially active substances and have led to the discovery of antibacterial agents like cephalosporins, ant diabetic agents like acarbose, and anticancer agents like epirubicin [46-50].

Natural products from marine organisms

The first active compounds to be isolated from marine species were spongouridine and spongothymidine from the Carribean sponge Cryptothecacryptain the 1950s. These compounds are nucleotides and show great potential as anticancer and antiviral agents [51-54]. Their discovery led to an extensive research to identify novel drug candidates from marine sources. About 70% of the earth's surface is covered by the oceans, providing significant biodiversity for exploration for drug sources. Many marine organisms have asedentary lifestyle, and thereby synthesize many complex and extremely potent chemicals as their means of defense from predators. These chemicals can serve as possible remedies for various ailments, especially cancer [26-35]. One such example is discodermolide, isolated from the marine sponge, Discodermiadissoluta, which has a similar mode of action to that of paclitaxol and possesses a strong antitumor activity. It also exhibits better water solubility as compared to paclitaxel [36-45]. A combination therapy of the two drugs has led to reduced tumor growth in certain cancers.

Objectives

To extract the crude drug from the leaves of Hibiscus Cannabis L. Plant.

To screen the anti-oxidant activity, anti-helminthic activity (which is published in paper –II of this research) of the crude extract using standard procedures.

Materials and Methods

Collection, Identification and Authentication of the Hibiscus cannabis L

The fresh leaves of the plant Hibiscus. Cannabis were collected from the surrounding areas of Injaram, East Godavari district, Andhra Pradesh. The plant was identified & authenticated by the Dr. M. Raghuram, Professor, Department of Botany& microbiology in Acharya Nagarjuna University, Guntur.

Extraction

The plant was collected and cleaned with water and the leaves were separated from the plant and the leaves are shade dried for a period of 3-4 days, then it was powdered & sieved with sieve no 44.

Extraction by maceration

In this process, the whole or coarsely powdered crude drug is placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved [9]. The mixture was strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing. Circulatory extraction.

The powdered plant material was weighed and the powder was placed in a conical flask and 50% hydro alcohol (ethanol: water 1:1) was added to it until the powder was fully soaked and 1ml of benzene was added to it to avoid microbial contamination and is allowed to stand for 48 hrs [10]. The powdered plant material was weighed and was placed in a conical flask and ethanol was added to it until the powder was fully soaked and allowed to stand for 48 hrs [11].

After 48hrs the mixture was filtered by using Buchner funnel, the filtrate containing drug and drug extract, the mixture was subjected to distillation process by the distillation process alcohol was separated out and the crude drug extracts using the specific solvents were separated, the hydro alcoholic extract contains water so it needs to be separated in order to concentrate the extract [12]. The water was removed by heating at 100°c in the hydro alcoholic extract ,the water was evaporated and the drug extract changes into thick viscous substance the crude drug substance was ready to use for the further tests and the hydro alcoholic and ethanolic extract obtained is evaluated for anti-helminthic activity.

Phytochemical evaluation

Test for flavonoids: Shinoda Test: To the extract, a few magnesium turnings and 1-2 drops of conc. HCl were added; red colour was observed indicates the absence of flavones [13].

To the extract, 10% sodium hydroxide or ammonia was added, dark yellow color was observed indicates the absence of flavones.

Test for glycosides: Legal's test: To the extract add 1ml of pyridine, 1ml of sodium nitroprusside, pink colour was not observed Indicates the absence of glycosides [14].

Test for alkaloids: Dragendroff's Test: In a test tube containing 1ml of extract, few drops of dragendroff's reagent was added, orange color was not observed Indicates the absence of alkaloids [15].

Test for Fixed oils: To 5 drops of sample added 1ml of copper sulphate solution then add 10%NaOH solution. A clear solution is obtained which shows glycerine is present in the sample. The cupric hydroxide formed in the reaction does not precipitate out as it is soluble in glycerine [16-19].

To 5 drops of the sample added a pinch of sodium hydrogen sulphate; pungent odour emanates indicating presence of glycerine [20].

Biological Evaluation

The extracted compound was subjected to investigate the following biological studies

Antioxidant Activity

Oxidative stress

Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. All forms of life maintain a reducing environment within their cells. This reducing environment is preserved by enzymes that maintain the reduced state through a constant input of metabolic energy. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA.

In humans, oxidative stress is involved in many diseases, such as atherosclerosis, Parkinson's disease, Heart Failure, Myocardial Infarction, Alzheimer's disease and chronic fatigue syndrome, but short-term oxidative stress may also be important in prevention of aging by induction of a process named mitohormesis. Reactive oxygen species can be beneficial, as they are used by the immune system as a way to attack and kill pathogens. Reactive oxygen species are also used in cell signaling. This is dubbed redox signaling.

Chemical and biological effects

In chemical terms, oxidative stress is a large rise (becoming less negative) in the cellular reduction potential, or a large decrease in the reducing capacity of the cellular redox couples, such as glutathione. The effects of oxidative stress depend upon the size of these changes, with a cell being able to overcome small perturbations and regain its original state. However, more severe oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis.

A particularly destructive aspect of oxidative stress is the production of reactive oxygen species, which include free radicals and peroxides. Some of the less reactive of these species (such as superoxide) can be converted by redox reactions with transition metals or other redox cycling compounds (including quinones) into more aggressive radical species that can cause extensive cellular damage. The major portion of long term effects is inflicted by the damage on DNA. Most of these oxygen-derived species are produced at a low level by normal aerobic metabolism and the damage they cause to the cells is constantly repaired. However, under the severe levels of oxidative stress that cause necrosis, the damage causes ATP depletion, and thus preventing the controlled apoptotic death and causing the cell to simply fall apart shown in Table:1.

Table 1: Oxidant and biological effects

S. No.	Oxidant	Description
1	$\bullet O_2-$, superoxide anion	One-electron reduction state of O_2, formed in many autoxidation reactions and by the electron transport chain. Rather unreactive but can release Fe^{2+} from iron-sulfur proteins and ferritin. Undergoes dismutation to form H_2O_2 spontaneously or by enzymatic catalysis and is a precursor for metal-catalyzed $\bullet OH$ formation.
2	H_2O_2, hydrogen peroxide	Two-electron reduction state, formed by dismutation of $\bullet O_2-$ or by direct reduction of O_2. Lipid soluble and thus able to diffuse across membranes.
3	$\bullet OH$, hydroxyl radical	Three-electron reduction state, formed by Fenton reaction and decomposition of peroxynitrite. Extremely reactive, will attack most cellular components.
4	ROOH, organic hydroperoxide	Formed by radical reactions with cellular components such as lipids and nucleobases.
5	$RO\bullet$, alkoxy and $ROO\bullet$, peroxy radicals	Oxygen centered organic radicals. Lipid forms participate in lipid peroxidation reactions. Produced in the presence of oxygen by radical addition to double bonds or hydrogen abstraction.
6	HOCl, hypochlorous acid	Formed from H_2O_2 by myeloperoxidase. Lipid soluble and highly reactive. Will readily oxidize protein constituents, including thiol groups, amino groups and methionine.
7	ONOO-, peroxynitrite	Formed in a rapid reaction between $\bullet O_2-$ and $NO\bullet$. Lipid soluble and similar in reactivity to hypochlorous acid. Protonation forms peroxynitrous acid, which can undergo homolytic cleavage to form hydroxyl radical and nitrogen dioxide.

Various models of antioxidant activity:

I. Nitric oxide radical scavenging activity method.
II. Scavenging of superoxide radical method.
III. Scavenging hydroxyl radical in deoxyribose method.
IV. Scavenging hydrogen peroxide radical method

V. Scavenging hydroxyl radical in p-NDA method.
VI. DPPH method

Here we followed two methods:

I. Nitric oxide radical scavenging activity:

Principle: Nitric oxide generated as a result of decomposition of sodium nitroprusside in aqueous medium, interacts with oxygen at physiological pH to produce nitrite ions, which are measured by using Griess reaction. In this reaction, nitrite ions were subjected to diazotization followed by azo coupling reactions to yield an azo dye. Absorbance of this dye was measured at 546nm.here the ability of the drug extract to inhibit nitric oxide generation from nitroprusside was evaluated by comparing with the control absorbance values. The reagents and he composition taken was shown in Table: 2.

Table 2: Reagents used and Composition

S. No.	Reagent	Composition
1	Griess Reagent	1%sulphanilamide, 2% ortho phosphricacid, and 0.1%naphtylethylenediamine dihydrochloride in 100ml water.
2	Phosphate buffer saline (PH 7.4)	2.3g of disodium hydrogen phosphate, 0.1g of potassium dihydrogen phosphate, 8g of NaCl, in 1000ml water.
3	Sodium nitroprusside solution	1%w/w solution (1gm/100ml water)

Preparation of working solutions:

Standard: ascorbic acid, standard phosphate buffer solution, 5mL of sodium nitroprusside solution, Griess' reagent was added.
Control: 5ml Dimethyl sulphoxide, standard phosphate buffer solution, 5mL of sodium nitroprusside solution, Griess' reagent were added.
Test: 5mL each of isolated plant derivative, standard phosphate buffer solution, 5mL of sodium nitroprusside solution, Griess' reagent were added.
Blank: Dimethyl sulphoxide

Calculation:

$$\%\text{INHIBITION} = \frac{\text{ABSORBANCE(BASAL CONTROL)} - \text{ABSORBANCE(SAMPLE)}}{\text{ABSORBANCE(BASAL CONTROL)}} \times 100$$

Procedure:

The nitric oxide radical scavenging activity was measured by using Griess' reagent.
1. 5mL each of isolated plant derivative, and ascorbic acid (standard) of different concentrations (25–200 μg/mL) in standard phosphate buffer solution (pH 7.4) were incubated with 5mL of sodium nitroprusside solution (5mM) in standard phosphate buffer (pH 7.4) at 25°C for 5 hours.
2. Control was prepared without compound but with equivalent amount of buffer and kept for incubation.
3. After incubation, 0.5mL of the incubation mixture was mixed with 0.5 mL of Griefss reagent.
4. Then absorbance was measured at 546nm against basal control.

5. From the absorbance the percent scavenging activity was calculated using the same formula as described above.

6. The experiments were performed in triplicate.

Results and Discussion

Anti-Oxidant Activity of the Isolated Extract of Hibiscus Cannabis

The isolated different extracts of the plant that is hydro alcoholic extract, ethanolic extract, water extract isolated from the plant Hibiscus Cannabis are screened for anti-oxidant activity by Nitric oxide method at the concentrations of 25µg/ml, 50µg/ml, 75µg/ml, and 100µg/ml. Standard used was Ascorbic acid, Control used was DMSO. The extracts of HE showed significant activity with remaining all compound were shown moderate activity when compared with standard Ascorbic acid. The results were shown in Table 3 Figure 1.

Table 3: Anti-oxidant activity of the plant extracts of Hibiscus Cannabis

Comp	%Scavenging activity [mean ± SEM] µ/ml			
	25µ/ml	50µ/ml	75 µ/ml	100µ/ml
HE	46**	56	64	87**
EE	33	48	75*	84*
WE	21	37	48	70*
Std	61	76	84	96

Where,

HE: Hydro alcoholic extract
EE: Ethanolic extract
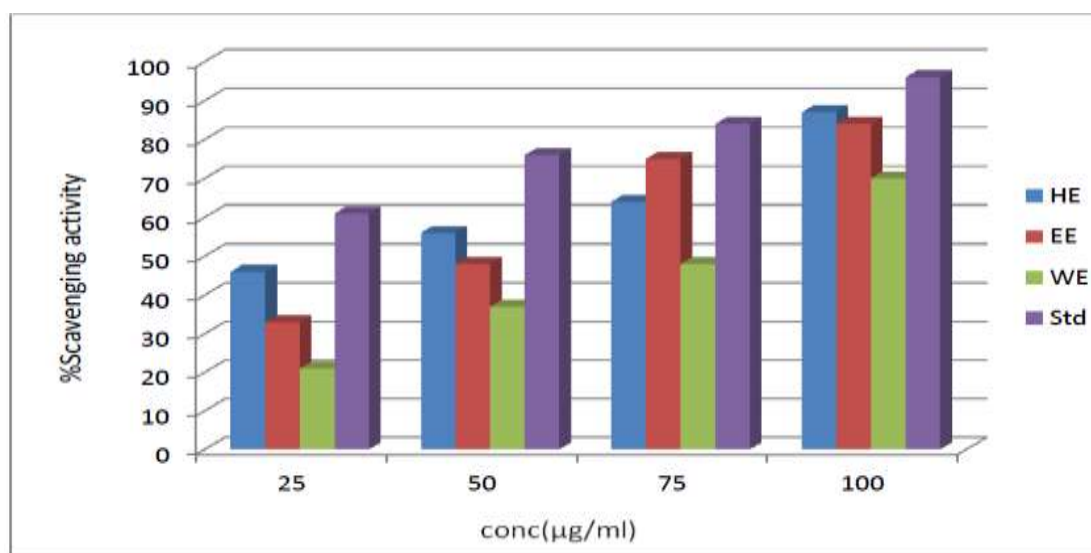WE: Water extract

Figure 1: Anti-oxidant activity of the plant extracts of Hibiscus Cannabis

*P<0.1, **P<0.01 compared with standard (Ascorbic Acid)

CONCLUSION

Finally we have demonstrated, that the hydro alcohol extract of the leaves of Hibiscus Cannabis extract showed potent activity when compared to the ethanolic and water extracts of the plant Hibiscus

Cannabis L and the solvent fractions exhibiting considerable activity (dose dependent) when compared with reference standard. The present research work showed the validity and the clinical use of hydro alcohol extract of Hibiscus Cannabis in the control of anti-helminthic and anti-oxidant activity. However further investigation required for chemical and pharmacological properties.

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