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R E S E A R C H A R T I C L E

In Vitro Antioxidant Study on Wound Healing Activity of *Hibiscus Cannabinus (Kenaf) L.*Seed extracts

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A B S T R A C T

Hibiscus cannabinus (Kenaf) is a potential source of bioactive constituents and natural antioxidant. The current study determined the impact of various solvents on extraction yield, recovery of polyphenol and flavonoid, antioxidant, anticancer, and antibacterial properties of Kenaf leaves and seed. The powder of leaves and seed was separately extracted with *n*-hexane, ethyl acetate, ethanol, and water solvent. Among them, the ethanol extract of leaves and seed showed the highest extraction yield, and their GC-MS analysis revealed a total of 55 and 14 bioactive compounds, respectively. The total polyphenols (TP) and flavonoids (TF) content were quantified by a spectrophotometric technique where water extracts displayed a noteworthy amount of TP and TF content compared to other extracts. A similar demonstration was noticed in antioxidant activity, evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) and hydrogen peroxide scavenging capacity. In addition, cytotoxicity and anti-lung cancer activity were identified against mouse embryonic fibroblast (NIH3T3) and human lung cancer (A549) cells.

Keywords: *Hibiscus cannabinus*, polyphenol, flavonoid, anti-lung cancer activity

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Traditional medicine is a term to used to describe ancient and cultural bound health practices that existed before the application of science to health matters in official modern scientific medicine or allopathic. In medicine, a wound is atype of injury in which skin is turn, cut or punctured on open or where blunt force trauma causes contusion. Pathology it specifically refers to a sharp injury which damages the dermis of the skin. Types of wound 1.open 2. closed. Kenaf (*Hibiscus cannabinus*) is an annual herbaceous dicotyledonous plant, belongs to the

Malvaceae family, is widely distributed in Asia and Africa, and grows mostly in temperate to tropical areas [8]. Kenaf (leaves and seed) has many significant medicinal properties, including anticancer, antioxidants, analgesic, anti-inflammatory, aphrodisiacs, and hepatoprotective activities [9,10]. In traditional medicine, Kenaf is used to treat various diseases; for instance, a paste of the leaf and stem is used to treat Guinea worms disease and anemia in Africa [10].

Moreover, in ayurvedic medicine, the leaves are used to treat various disorders, such as of the blood, diabetes, bilious, the throat, and coughs[10,11].These medicinal benefits are exposed due to the presence of abundant phenyl propanoid compounds in the Kenafplant[13].

2. Materials and Methods

Acute oral toxicity study was performed as per OCT423 guidelines (acute toxic class method) Wister Rats of either sex select by random sampling technique was used for the study. The animal we are kept fasting for overnight providing only water, after which the plant extract was administered orally and the objects of 14 days. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If the mortality was not observed, the procedure was repeated for further doses.

Preparation of the Active Formulation

Oily phase which consisted of Olive oil and surfactant (Abil EM 90) was heated up to 75°C 1°C. Lipacide® was added when the temperature was acquired. At the same time, aqueous phase consisting of water and magnesium sulphate was heated to the same temperature. Vitamin C was added finally to the aqueous phase. Aqueous phase was added to the oil phase drop by drop. Stirring was continued at 2000 rpm by the mechanical mixer for about 25 minutes, until the emulsion cooled to room temperature. In the next step, multiple emulsions were prepared. Aqueous phase consisted of Synperonic®, the hydrophilic emulsifier, and water. Triethanolamine was added to adjust the pH of the emulsion. PE was added slowly to the aqueous phase at 700 rpm in 20 minutes. After the complete addition of the PE, the speed of the mixer was reduced to 400 rpm for homogenization, for a period of 35 minutes.

$$\text{Radicals cavenging activity (\%)} = \frac{\text{OD control} - \text{OD control}}{\text{OD control}} \times 100$$

Ic50, which is the concentration of the sampler quired to scavenge 50% of free radicals was calculated. Scavenging of ABTS radical action ABTS assay is relatively recent one, which involves a more drastic radical, chemically produced and, is often used for screening complex antioxidant mixture such as plant extracts, beverages and biological fluids. The solubility in both the organic and aqueous media and the stability in a wide pH range raised the interest in the use of ABTS e+ for the estimation of the antioxidant activity (Nenadis et al.,2004).

Preparation of extracts and standard solutions:

Accurately weighed 13.5mg of each of the extracts and standard, ascorbic acid are dissolved separately in 2ml of freshly distilled DMSO. These solutions were serially diluted with freshly distilled DMSO to obtain the lower dilutions.

Procedure:

ABTS (54.8 mg, 2 mM) was dissolved in 50 ml of distilled water and potassium persulphate 10.3 ml, 17 mM,) was added. The reaction mixture was left to stand at room temperature overnight in dark before usage. To 0.2 ml of various concentrations of the extracts/standard added 1.0 ml of freshly distilled DMSO and 0.16ml of ABTS solution to make a final Volume of 1.36 ml. After 20 min, absorbance was measured spectrophotometrically at 734um (Re et al., 1999).

Scavenging of hydrogen peroxide:

Hydrogen peroxide is generated in-vivo by several oxidase enzymes. There is increasing evidence that hydrogen peroxide, either directly or indirectly via its reduction product hydroxyl radical (OH) causes severe damage to biological systems. In this method, when a scavenger is incubated with hydrogen peroxide, the decay or loss of hydrogen peroxide can be measured spectrophotometrically at 230nm (Jayaprakash et al.,2004).

Preparation of extracts and standard solutions

Accurately weighed 30mg of the extracts and standards, rutin and ascorbic acid were dissolved separately in10 ml of methanol. These solutions were serially diluted with methanol to obtain the lower dilutions.

Procedure

A solution of hydrogen peroxide (20mM) was prepared in phosphate buffered saline (PBS, pH 7.4). Various concentrations of 1 ml of the extractor standard in methanol were added to 2ml of hydrogen peroxide solution in PBS. After10min, the absorbance was measured at 230 nm (Jayaprakasha et al., 2004).

Scavenging of super oxide radical by alkaline DMSO method in alkaline DMSO method, superoxide radical is generated by the addition of sodium hydroxide to air saturated dimethyl sulfoxide (DMSO).

The generated superoxide remains stable in solution, which reduces nitro blue tetrazolium into formazan dye at room temperature and that canbemeasuredat560 nm. Superoxide scavenger capable of reacting inhibits the formation of areddyeformazan (Elizabeth and Rao, 1990)

Wound Healing Activity

Procedure:

2.5 gm of crude extract and 15gm of hard paraffin were weighed and transferred into a in a dish and melted in a water bath, 25gm of cetostery lalcohol was added followed by 10 gm white bees wax and 450gm of white soft paraffin were mixed at a temperature of 70°c till it solidify.

3. Results and Discussion

Acutetoxicity studies:

On the basis of toxicity studies it was observed that the ethanolic and acqeous extract of hibiscus cannabinus were

non toxic and didn't Induce death at the highest single dose 2000mg/kg B.w. no toxic symptoms like behavioral changes, locomotion, convulsions etc were observed.

In-vitro antioxidant activity:

Ethanolic extract exhibited potent in vitro antioxidant activity in scavenging of ABTS radial cation with IC_{50}value, 5.37± 0.20/ml. The result was found to be high value when compared to standard drug. Aqueous extract showed moderate antioxidant activity with IC_{50} value 56.03±0.1/ml when compared to standard drug. In hydrogen peroxide method ethanol extract showed potent activity with IC_{50} value,25.89±0.14/ml as compared to standard drug where as aqueous extract has shown less activity with IC_{50}, 167.14±0.33value. In alkaline DMSO methods, since IC_{50} values were found to be greater than 1000 microgram per ML both extract were formed to be in active.

S.NO	0th day	4th day	8th day	16th day
CONTROL	514.12 ± 0.76	411.26 ± 3.34	402.36 ± 1.16	359.81 ± 0.62
STANDARD	511.62 ± 1.52	326.62 ± 1.45	134.67 ± 0.39	15.37 ± 0.91
EXTRACT (100 mg)	507.00 ± 2.86	465.67 ± 1.85	276.27 ± 0.83	72.02 ± 0.72
EXTRACT (200 mg)	509.82 ± 1.62	442.36 ± 1.32	259.27 ± 0.62	52.27 ± 0.94

Fig.1. Effect of Hibiscus cannabinus leaf extract on excision wound model (%wound closure)

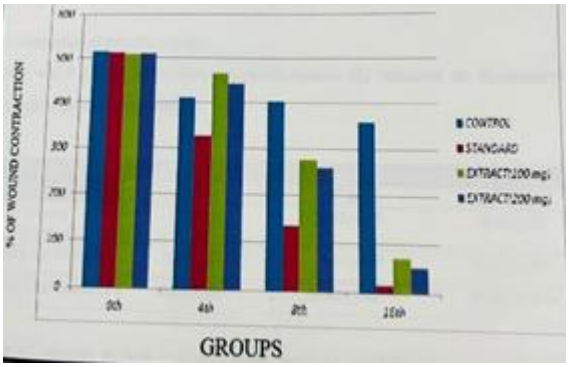

Fig.2. Percentage of wound contraction Estimation of protein content

Fig.3

Discussion:

Hibiscus cannabinusis a large woody shrub with many-branched. The ethanol extract of Hibiscus cannabinus seeds showed strong by inhibiting ABTS radical action and hydrogen peroxide scavenging activities when compared with standards such as ascorbic acid and Rutin. In addition, the Ethanolic extract found to contain a noticeable amount of total phenols, which play a major role in controlling antioxidants. The topical application of Hibiscus cannabinus is ointment increased the percentage of wound contraction and this indicates rapid epithelization and collage nation. Wound healing process consists of different phases such as granulation, collage nation, collagen maturation and scar maturation which are concurrent but independent of each other.

It is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissue as close as possible to its normal state. Wound contracture is a process, commencing in the fibroblastic stage where by the area of the wound undergoes shrinkage. Collagen, the major component which strengthens and supports extracellular tissue is composed of amino acids, hydroxyl proline, which has been used as a biochemical marker for tissue collagen (KumarR,2006)

Hence in this investigation to models were used to assess the effect of the Ethanolic extract of Hibiscus cannabinus seeds. The result of the present investigation showed that a Hibiscus cannabinus possess a definite prohealing action.

4. Conclusion

Considering the rich diversity of plants, it is expected that screening and scientific evaluation of plant extracts of their wound healing activity may provide new antiseptic and antimicrobial substances. Hence in the present investigation the wound healing activity of hibiscus cannabinus seeds of both aqueous and ethanolic has been demonstrated for the first time against wound healing by the 2 methods. Thus, these plants can be utilised as an alternative source of useful drugs. Further studies are needed with this plant to isolate, characterize and elucidate the structure of the bioactive compounds of this plant for industrial drug formulation.

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