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Study of Phytochemical Constituents of Hydro-Alcoholic Extract of Hibiscus Cannabinus L.

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Abstract:

The concept that immune responses are modulated to alleviate diseases has existed in many forms of traditional medicine beliefs, with plants being used in such systems to promote health and to maintain the body's resistance against infections by potentiating immunity. Some of these plants are specifically the extracts and some of the constituents of dried leaves of *Hibiscus Cannabinus Linn* (family Malvaceae) have been reported to possess impressive medicinal property in previous study. The present study was undertaken to evaluate the phytochemical constituent of hydro-alcoholic extract of *Hibiscus Cannabinus Linn* leaves on the biological system to substantiate the traditional claim. The study helps in understanding the potential constituent of *Hibiscus Cannabinus Linn* leaves on the biological system in respect to biochemical consequences recovery and immune system. This study was therefore designed to evaluate the phytochemical constituent of the crude hydro-alcoholic extract of the dried leaves of Hibiscus cannabinus Linn.

Keywords: Medicinal plant, Phytochemical, Hibiscus, Oil extract.

Introduction:

Medicinal plants are of great importance to the health of individuals and communities [1]. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds [1, 2]. Many of these natural products have vital roles as mediators of ecological interactions; that is, they have functions in ensuring a continued survival of particular organisms in often hostile environments where there is competition with other organisms [2]. Traditional medicinal treatment in India, Ayurved, used several herbs in the form of powder, mixture, tablet, extract, syrups, kadha (concentrated extract) and asavas (fermented extracts) [3]. These preparations addressed several medical problems in reproducible manner, rapidly or at times slowly, presumably due to differences in there amount of bioactive molecules. Medicinal plants are generally used in traditional medicine for the treatment of many ailments [4, 5]. In developing countries, the use of medicinal plants in the treatment of infectious disease is rife and reasons include the high cost of effective drugs [6]. However, potential indigenous plants exploited for medicinal purposes have to undergo basic phytochemical screening and bioassay as first step towards the ultimate development of drugs [7].

One such tropical medicinal plant *Hibiscus Cannabinus Linn.*, commonly known as kenaf, (locally known as *Ambadi* in Marathi or *Ambashtha* in Sanskrit) [3], belonging to family Malvaceae is used in India and many other countries. Its leaves are eaten as vegetable, dry flowers are used to make syrup and vegetables in Maharashtra [8, 9] and some have been reported to be used as medicine in Ayurveda [3]. Therefore, the present study aims at investigating the presence of phytochemical constituents in this potential plantas a source of nutritional and therapeutic purposes.

Material and methods:

Collection and preparation of plant materials

Fresh leaves and flower of H. Cannabinus Linn were collected from uncultivated farmlands located near Nagpur region of Maharashtra state during rainy session from August to October. The plant samples were identified by a botanist from University Department of Botony, RTM Nagpur University. The voucher specimens (no.9621 dated 30/06/2011) were deposited in the Biochemistry Department of RTM Nagpur University, Nagpur. Special recommendation for use of *Hibiscus cannabinus L.* was taken from National Medicinal Plant Board, Department of AYUSH, Ministry of Health and Family Welfare, Government of India through Department of AIDS control, National AIDS Control Organization (NACO), Ministry of Health and Family Welfare, Government of India. Fresh plants of *Hibiscus Cannabinus L.* were washed with plain water followed by distilled water, dried under shed, powdered in grinding mill, and stored in air tight zip bags. Powdered leaves (500 g) and flower (500g) was packed in soxhlet apparatus separately. The drug was defatted with petroleum ether (60-80°C) for about 10-12 complete cycles. Defatted material was extracted with water: alcohol (1:1 ratio) in soxhlet apparatus for both leaves and flower. This dried crude extract of water: alcohol fraction of leaves and flower were stored in desiccators and used for further experiment. Aqueous and 95 % ethanol extract was prepared by above method of soxhlet apparatus where solvent system was used as distil water in case of Aqueous extract and 95% ethanol was used for ethanol extract.

Oil extraction from dried leaves and flower of *Hibiscus Cannabinus Linn.*

40 g of the powdered leaves and flower were weighed separately, tied up in filter papers and put into the thimble. A reflux condenser and a round bottomflask were fitted above and below the thimble respectively. This Soxhlet extractor, was clamped firm into position and 250 ml of petroleum ether poured into the round bottom flask petroleum ether was preferably used in place of water: alcohol for the extraction of *Hibiscus cannabinus L.* oil from leaves and flower. Petroleum ether is more selective to-wards true lipids. The Soxhlet extractor was then heated electrically on a heating mantle. Continuous extraction was carried out for a period of 8 h with about eighteen

refluxes. The samples were then removed and the petroleum ether was evaporated. The oils were poured into bijou bottles and the flasks washed with some quantity of petroleum ether and transferred into the bottles. These were later evaporated on the heating mantle and stored in a refrigerator. The weights of the oils obtained were recorded and used for percentage yield calculations.

Partitioning of essential oils

The crude oil from leaves and flower extracts were partitioned according to the methods of Edeoga et al. (2005) [10].The oil was poured into a clean dry separating funnel. 10 ml of aqueous (50%) ethanol was added into the separating funnel and additional 40 ml of aqueous ethanol was later added. 50 ml of organic solvent (chloroform-ether mixture) was added.

The mixture was then shaken vigorously and allowed to stand for about 30 min to partition. The two fractions were separated and put into two conical flasks and evaporated to dryness. The organic phase, which appeared above, was labeled as organic while the phase below was labeled as aqueous. Same procedure was followed for partitioning of oil from leaves and flower extract.

Phytochemical Screening -

The preliminary phytochemical analysis of the hydro-alcoholic leaves extract of *Hibiscus Cannabinus l.* was revealed for the presence of varying amount of alkaloids, saponins, tannins, anthraquinones, cardiac glycosides, cardenoledes, flavonoids and phlobatanins. Chemical tests were carried out on the hydro-alcoholic extracts to identify the constituents using standard procedures as described by Sofowora (1993) [11], Trease and Evans (1989) [12] and Harborne (1973) [13].

Test for tannins

About 2 ml of the aqueous extract was stirred with 2 ml of distilled water and few drops of FeCl3 solution were added. The formation of a green precipitate was an indication for the presence of tannins.

Test for saponins

5 ml of aqueous extract was shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication for the presence of saponins.

Test for phlobatannins

About 2 ml of aqueous extract was added to 2 ml of 1% HCl and the mixture was boiled. Deposition of a red precipitate was taken as an evidence for the presence of phlobatannins.

Test for flavonoids

To 1 ml of aqueous extract was added 1 ml of 10% lead acetate solution. The formation of a yellow precipitate was taken as a positive test for flavonoids.

Tests for anthraquinones

(a) Borntrager's test: 3 ml of aqueous extract was shaken with 3 ml of benzene, filtered and 5 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of a pink, red or violet colour in the ammonical (lower) phase indicates the presence of free anthraquinones.

(b) 3 ml of the aqueous extract was boiled with 3 ml of aqueous sulphuric acid and filtered while hot. 3 ml of benzene was added to the filtrate and shaken. The benzene layer was separated and 3 ml of 10% NH3 added. A pink, red or violet colouration in the ammonical (lower) phase indicates the presence of anthraquinone derivatives.

Test for terpenoids

2 ml of the organic extract was dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. A greyish colour indicates the presence of terpenoids.

Tests for steroids

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

(ii) The development of a greenish colour when 2 ml of the organic extract was dissolved in 2 ml of chloroform and treated with sulphuric and acetic acids indicates the presence of steroids.

Test for alkaloids

3 ml of aqueous extract was stirred with 3 ml of 1% HCl on a steam bath. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Tests for carbohydrates

(a) Molisch's test: 3 ml of the aqueous extract was added to 2 ml of Molisch's reagent and the resulting mixture shaken properly. 2 ml of concentrated H_2SO_4 was then poured carefully down the side of the test tube. A violet ring at the interphase indicates the presence of carbohydrate.

(b) To 3 ml of the aqueous extract was added about 1 ml of iodine solution. A purple colouration at the interphase indicates the presence of carbohydrates.

Tests for glycosides

(a) Liebermann's test: 2 ml of the organic extract was dissolved in 2 ml of chloroform and 2 ml of acetic acid was added and the solution cooled well in ice. Sulphuric acid was then added carefully. A colour change from violet to blue to green indicates the presence of a steroidal nucleus (that is, a glycone portion of glycoside).

(b) Salkowski's test: 2 ml of each extract was dissolved in 2 ml of chloroform. 2 ml of sulphuric acid was added carefully and shaken gently. A

reddish brown colour indicates the presence of a steriodal ring (that is, a glycone portion of glycoside).

(c) Keller-Kiliani test: 2 ml of each extract was dissolved in 2 ml of glacial acetic acid containing one drop of FeCl3 solution. The mixture was then poured into a test tube containing 1 ml of concentrated H_2SO_4. A brown ring at the interphase indicates the presence of a deoxy sugar, characteristic of cardenolides.

Reducing Sugar

To 0.5 ml of extracts solution, 1ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitate.

Result and discussion:

The physical appearance of extract i.e. colour, consistency and yield are given in table 1. The phytochemical characteristics of the four medicinal plants investigated are summarized in Table 2. The results reveal the presence of medicinally active constituents in the plant studied.

Quantitative estimation of the percentage yield of the oil extracts from the four medicinal plants studied is summarized in Table 3.

Qualitative Screening of phytochemical

Fluorescence analysis of powder

The fluorescence character of powdered leaves of *Hibiscus Cannabinus Linn* was studied both in day light and UV light. (Table 3)

The present study investigated the phytochemical constituent of 95% ethanol, aqueous and hydroalcoholic extracts of *H. Cannabinus Linn* leaves. The results indicated presence of alkaloids and tannins in aqueous extract. Alkaloids are known to have antimicrobial, antifungal and anti-inflammatory effect [5], claiming the result presented in our previous publication [14] and it also acts as an anti-hypertensive agent [11]. Anthraquinone tested positive in hydro alcoholic extract. Anthraquinones can induce laxative effect [15] and hence, the use of H. Cannabinusas laxative and nervous system depressant may result from the presence of anthraquinones. Flavonoids and phenols were abundant in aqueous extract and hydro-alcoholic extract than 95% ethanol extract. These are potent water soluble antioxidants which prevent oxidative cell damage suggesting antiseptics, anticancer, anti-inflammatory effects and mild anti-hypertensive properties [16, 5].

Furthermore, plant phenolics are major group of compounds acting as primary antioxidants or free radical scavengers [17]. The therapeutic potential of antioxidants in controlling degenerative diseases with marked oxidative damage from reactive oxygen species or free radicals have been reported [18, 19, 20].

All extracts of *H. Cannabinus L.* with high phenolic content showed higher antioxidant and inhibition of lipid peroxidative activity. These suggest

its potential in the treatment and prevention of various oxidative related diseases. Therefore, hydro-alcoholic of *H. Cannabinus L.* could be exploited as sources of free radical scavengers and bioactive metabolites for nutritional, medicinal and commercial purposes.

Table 1: Colour, Consistency and yield of the extract and fraction

Name	Colour	Consistency	Yield
Hydro- alcoholic HC leaves extract	Dark green	Sticky slurry	16.5
95% ethanol flower extract	Dark brown	Sticky slurry	09.1

Table 2: Qualitative Screening of Phytochemicals of herbal plant *Hibiscus Cannabinus Linn* leaves extract

Sr. No	Test	Aqueous extract	95% Ethanol extract	Hydro-Alcoholic extract
1	Alkaloids	+	+	+
2	Carbohydrates	+	+	+
3	Protein	-	+	+
4	Glycoside	+	-	+
5	Steroids and Sterols	-	+	+
6	Anthraquinones	-	-	+
7	Flavonoids	+	-	+
8	Tannins and Phenol compounds	+	+	+
9	Saponin Test	-	+	+
10	Fixed Oil	-	+	+

(+) Present, (-) Absent

Table 3: Fluorescence Analysis of powder

Sr. No	Drug	UV Light	Visible Light
1	1 N NaOH	Yellow	Yellow
2	Ammonia	Greenish yellow	Green
3	1 N HCL	Light Broun	Green
4	50 % HNO_3	Reddish brown	Green
5	Only powder	Green	Green

Table 4: Quantitative Screening of Phytochemicals of herbal plant *Hibiscus Cannabinus Linn* leaves extract

Sr. No.	Extracts and fractions	Phenol content (% w/w)	Tannin content (% w/w)	Anthocyanidin content (% w/w)
1	Hydro-alcoholic Leaves extract	66 µg/ml	0.334 µg/ml	34 µg/ml
2	Hydro-alcoholic Flower extract	74 µg/ml	0.194 µg/ml	10.2 µg/ml

Table Shows quantitative screening of phytochemicals of hydro-alcoholic extract of *H. Cannabinus Linn* leaves.

References:

1) **Edeoga H. O., Okwu D. E., Mbaebie B. O. (2005)** Phytochemical constituents of some Nigerian medicinal plants, Afr. J. Biotechnol. 4(7): 685-688.

2) **Mann J. (1978).** Secondary Metabolism. Oxford University Press, London, p. 154.

3) **Bapalal C. Nighantu (1986)** Adarsha. Varanasi VidyaBhavan Ayurveda, Granthamala, Varanasi, pp. 100-110.

4) **Njoku P. C. and Ezeibe A. U. (2007).** Phytochemical and elemental analyses of *Helianthus annus*and its use as blood clotting agent. J. Chem. Soc. Niger. 32(2): 128-132

5) **Ogukwe C. E., Oguzie E. E., Unaegbu C. and Okolue B. N. (2004).** Phytochemical screening on the leaves of *Sansevieria trifasciata*, J. Chem. Soc. Nigeria, 29(1): 8-10.

6) **Okeke I. N., Lamikanra A. and Edelma R. (1999).** Socioeconomic and behavioural factors leading to acquired bacterial resistance to antibiotics in developing countries. Emerg. Infect. Dis., 5: 18-27.

7) **Odebiyi A. and Sofowora A. E. (1978).** Phytochemical screening of Nigerian Medical Plants. Part II, Lloydia, 41: 234-246.

8) **Mabberly D. J. (1987)** The Plant Book-A portable dictionary of the higher plants, Cambridge University Press, Cambridge, pp. 706

9) **Jagtap S. D. (2005)** Ethnobotanical studies on tribal communities of Western and Eastern Satpura region of Maharashtra with aid of Pharmacognosy, PhD. Thesis, Pune University, Pune,

10) **Edeoga H. O., Okwu D. E. andMbaebie B. O. (2005).** Phytochemicalconstituents of some Nigerian medicinal plants, Afr. J. Biotechnol.4(7): 685-688.

11) **Sofowora A. (1993).** Medicinal Plants and Traditional Medicine in Africa.

12) Spectrum Books Ltd., Ibadan, Nigeria, pp. 191-289.

13) **Trease G. E. and Evans W. C. (1989).** Pharmacognosy, 11th edn., Bailliere Tindall, London pp. 45-50.

14) **Harborne J. B. (1973).** Phytochemical Methods. Chapman and Hall Ltd., London pp. 49-188.

15) **Karale K. (2013)** Antimicrobial potential of *Hibiscus Cannabinus Linn* Extract against opportunistic infection during HIV/AIDS Asiatic Journal of Biotechnology Resources MDIMCE - Volume 4; Issue 3,pp 18-24.

16) **Muller-Lissner S. A. (1993).** Adverse effects of Laxatives facts and fiction. Pharmacol., 47(1): 138-145.

17) **Del-Rio A., Obdulio B. G., Castillo J., Marrin R. R. and Ortuno A. (1997).** Uses and properties of citrus flavonoids. J. Agric. Food Chem., 45: 4505-4515.

18) **Kahkonen M. P., Hopia A. I., Vuorela H. J., Rouha J. P., Pihlaja K., Kujala T.S. andHeinonen M. (1999).** Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem., 47: 3954-3962

19) **Larson R. A. (1988).** The antioxidants of higher plants. Phytochem., 27(4): 969-978.

20) **Tripathi K. D. (1999).** Essential of Medical Pharmacology 4th ed. Jaypee Brothers Medical Publishers (P) Ltd. New Delhi, India. p.432.

21) **Vani T., Rajani M., Sarkar S. and Shishoo C. J. (1997).** Antioxidant properties of the ayurvedic formulation Triphala and its constituent. Int. J. Pharmacog., 35(5): 313-317.