PHYTOCHEMICAL SCREENING AND PHARMACOLOGICAL SCREENING OF COMMIPHORA *WIGHTII* (ARN.) BHANDARI.

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Abstract

Commiphora wightii (Arn.) Bhandari is a well known herbal plant of *Burseraceae* family. It is widely distributed throughout india and adjacent dry regions.. In India it is found in Rajasthan, Gujarat and Maharashtra. *C. wightii* are known to contain chemical constituents belonging to different chemical groups, namely, glycosides, alkaloids, steroids, terpenoids, flavonoids, tannins, coumarins and anthraquinones. In the present study we performed the phytochemical screening and pharmacological screening for hyperlipidemia..Preliminary phytochemical screening of methanolic stem bark extract revealed that the presence of all phytochemical except proteins, amino acids, saponins, oils and fats. In the petroleum ether extract only carbohydrates, alkaloids, glycosides and phytosterol were present. The results of Pharmacological screening showed that administration of methanolic extract of *Commiphora Wightii* at dose level 500mg/kg was effective for hypolipidemic condition.

Keywords: *Commiphora wightii*, phytochemical screening, bark extract, petroleum ether.

Introduction

Medicinal plants contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various ailments [1-3] reported that plants contain a wide variety of active principles. Phytochemicals are chemical compounds formed during the plants' normal metabolic processes and often referred to as "secondary metabolities" of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids [4] . The qualitative and quantitative estimation of the phytochemical constituents of a medicinal plant is considered to be an important step in medicinal plant research [5]. Phytochemical progress has been aided enormously by the development of rapid and accurate methods of screening plants for particular chemicals [6] . There are several standard methods used for the phytochemical screening of medicinal plants. There are variety of phytoconstitutents like alkaloids, steroids, saponins [7], phenolics [8] , flavonoids [9], saponins and cardiac glycosides[10] and tannins [11]. *C. wightii* (Arn.) Bhandari is a well known herbal plant of Burseraceae family. is commonly known as "Indian bedellium", "Mukul myrrh

tree", "Gugal", "Gugulu or "Guggul" in India. It is widely distributed in tropical regions of Africa, Madagascar and Asia. In India it is found in Rajasthan, Gujarat and Maharashtra [12] It is used in the Allopathic, Ayurvedic and Unani systems of medicines due to its anti-inflammatory, anti-rheumatic, hypocholesteremic and anti-fertility activities [13] (C. *wightti* yields guggul, an important oleo-gumresin which is complex mixture of resin (61 %), gum (29.3 %) and other chemicals (6.1 %) and used as incense, fixative in perfumery and in medicine [14] Since the *C. wightii* are known to contain chemical constituents belonging to different chemical groups, namely, alkaloids, glycosides, steroids, terpenoids, flavonoids, coumarins, tannins, and anthraquinones [15]. It is important to distinguish and determine the presence of major chemical groups employing simple chemical tests using various reagents. In the present study we performed the phytochemical screening and Pharmacological screening for hyperlipidemia of *C. wightii* .

Pharmacological screening for hyperlipidemia.

Material and Methods

Collection and preparation of plant material

The stem barks of *Commiphora wightii* were collected from local market of Bhopal. The plant was washed, chopped in to small pieces and dried under shade then powdered coarsely with a mechanical grinder. The powder was passed through sieve No. 40 and stored in an airtight container for further use. The stem bark of plant were authenticated by Dr. S. N. Dwivedi, Professor & Head, Janata PG College, APS University, Rewa (M.P.). Voucher specimen No. J/Bot.CWST-0185 was assigned.

Extraction of Plant Material

About 200 gm of coarsely powdered plant material was successively extracted by Soxhlet extraction method using solvents with increasing polarity viz. petroleum ether and methanol. Each time before extracting with next solvent, the powdered material was dried in hot air oven (mentioned temp range). Each extract was then concentrated by distilling off the solvent by evaporation to water bath. All the extracts thus obtained were stored in air-tight bottles at 4ºC for further experiments [16, 17]

Preliminary phytochemical screening

The extracts obtained from successive solvent extraction were then subjected to various qualitative chemical tests to determine the presence of various phyto constituents. Phytochemical screening of plant extracts was done by standard procedure [16]. The following tests were performed on extracts to detect various phyto constituents present in them.

(a) Detection of Carbohydrates

***Molish Test*:** 2 mL of aqueous extract was treated with 2 drops of alcoholic α-naphthol solution in a test tube, the mixture was shaken well and then 1mL of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand. Formation of violet ring at the junction indicated the presence of carbohydrates.

Fehling's test: 1mL of filtrate was boiled on water bath with 1 mL each of Fehling solutions A and B. Formation of red precipitate indicated the presence of sugar.

Barfoed's test: To 1 mL of filtrate, 1 mL of Barfoed's reagent was added and heated on boiling water bath for 2 min. Red ppt indicated presence of sugar.

Benedict's test: To 0.5 mL of filtrate, 0.5 mL of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 min. A characteristic colored precipitate indicated the presence of sugar.

(b) Detection of proteins and amino acids

Millon's test: To 2 mL of filtrate, few drops of Millon's reagent were added. A white precipitate which on heating turned to brick red indicated the presence of proteins.

Biuret test: The extract was treated with 1 mL of 10% sodium hydroxide solution in a test tube and heated. A drop of 0.7% copper sulphate was added to the above mixture. The formation of violet or pink colour indicated the presence of proteins.

Ninhydrin test: Two drops of ninhydrin solution were added to two mL of aqueous filtrate. A characteristic purple colour indicated the presence of amino acid.

(c) Detection of Alkaloids

Mayer's Test: To a few mL of filtrate, a drop or two of Mayer's reagent were added by the side of the test tube. A white or creamy precipitate indicated the test as positive.

Wagner's test: To a few mL of filtrate, few drops of Wagner's reagent were added by the side of the test tube. A reddish – brown precipitate confirmed the test as positive.

Hager's test: To a few mL of filtrate 1 or 2 mL of Hager's reagent (saturated aqueous solution of picric acid) were added. A prominent yellow precipitate indicated the test as positive.

Dragendorff's test: To a few mL of filtrate, 1-2 mL of Dragendorff's reagent was added. Formation of red precipitate indicated the test as positive.

(d) Detection of glycosides

For detection of glycosides, 50 mg of extract was hydrolysed with concentrated hydrochloric acid for 2 hours on water both, filtered and the hydrolysate was subjected to the following tests.

Borntrager's test: To 2 mL of filtered hydrolysate, 3 mL of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added to it pink colour indicated the presence of glycosides.

Legal's test: 50 mg of the extract was dissolved in pyridine, sodium nitroprusside solution was added and made alkaline using 10 % sodium hydroxide. Presence of glycoside was indicated by pink colour.

(e) Detection of saponins

Foam Test:: The extract (50 mg) was diluted with distilled water and made up to 20 mL. The suspension was shaken in a graduated cylinder for 15 min. A two cm layer of foam indicated the presence of saponins [16].

(f) Detection of phytosterol (Terpenoids and Steroids)

Libermann-Burchard's test: The extract was treated with chloroform. To this solution few drops of acetic anhydride were added, boiled and cooled. Concentrated sulphuric acid was added through the sides of test tube. An array of colour changes showed the presence of phytosterols.

Salkowski's Test: The extract was treated with chloroform and filtere. The filtrate was added with few drops of concentrated sulphuric acid, shaken and allowed to stand. If the lower layers turns red, sterol are present. Presence of golden yellow layer at bottom indicates the presence of triterpenes.

(g) Detection of fixed oils and fats

Spot test: A small quantity of extract was pressed between two filter papers. Oil stain on the paper indicated the presence of fixed oil.

Saponification test: A few drops of 0.5 N alcoholic potassium hydroxide solution was added to a small quantity of extract along with a drop of phenolphthalein. The mixture was heated on water bath for 2 hours. Formation of soap or partial neutralization of alkali indicated the presence of fixed oils and fats.

Solubility test: (a) To 2-3 mL of the alcoholic solution of extract, add few mL of chloroform and solubility was observed. (b) To 2-3 mL of the alcoholic solution of extract, add few mL of 90% ethanol and solubility was observed.

(h) Detection of phenolic compounds and tannins

Ferric chloride test: The extract (50 mg) was dissolved in 5 mL of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

Gelatin test: The extract (50 mg) was dissolved in 5 mL of distilled water and 2 mL of % solution of gelatin containing 10 % sodium chloride was added to it. White precipitate indicated the presence of phenolic compounds.

Lead acetates test: The extract (50 mg) was dissolved in distilled water and 3 mL of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenol compounds.

(i) Detection of flavonoids

Alkaline reagent test: An aqueous solution of the extract was treated with 10% ammonium hydroxide solution. Formation of Yellow fluorescence which becomes color less on addidition of few drops of dilute acid indicated the presence of flavonoids.

***Shinoda test* (Magnesium and hydrochloric acid reduction):** The extract (50 mg) was dissolved in 5 mL of alcohol and few fragments of magnesium ribbon and concentrated hydrochloric acid (Drop wise) were added. Presence of flavanol glycosides was inferred by the development of pink to crimson colour.

Lead Acetate Test: The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate indicated the presence of flavonoids.

Pharmacological Screening of Extract for Hyperlipidemia

Experimental animals

Wistar rats weighing 130-165g were used in the present study. The experimental animals were maintained under standard laboratory conditions in an animal house approved by the committee for the purpose of control and supervision on experiments on animals (CPCSEA) under 12 h light/dark cycle and controlled temperature (24 ± 2°C) and fed with commercial pellet diet and water *ad libitum*. All animals were acclimatized to the laboratory environment for at least one week before the commencement of experiment. The experimental protocol was approved by the Institutional Animal Ethical Committee, SRK University, Bhopal, Madhya Pradesh, India.

SCREENING MODELS

1: Diet-induced hyperlipidemic model

The animals were selected, weighed then marked for individual identification. Rats were made hyperlipidemic by the oral administration of atherogenic diet for 20 days. The rats were then given plant extracts suspended in 2% acacia at the dose of 200mg/kg b.w. once daily in the morning through gastric intubation for 14 consecutive days.

During these days, all the groups also received atherogenic diet in the same dose as given earlier. The control animals received the hyperlipidemic diet and the vehicle. At the end of treatment period, the animals were used for various biochemical parameters. Blood was collected by heart puncturing of rat under ether anesthesia and centrifuged by using centrifuge at 2000 rpm for 30 minute to get serum.

2: Triton-induced hyperlipidemic model

Animals kept for fasting for 18 h, will be injected a saline solution of Triton(Triton x-100) at the dose of 100mg/kg b.w. intra-peritoneally. The plant extracts, at the dose of 200mg/kg b.w., was administered orally through gastric intubation. The first dose being given immediately after triton injection and second dose 20 h later and continue the extraction process for 7 days. After 7 days dose the animals were used for various biochemical parameters. Blood was collected by heart puncturing of rat under ether anesthesia and centrifuged by using centrifuge at 2000 rpm for 30 minute to get serum. [18-19].

Collection of blood samples

Blood samples (0.2 ml) were collected serially through retro orbital puncture at 15 & 24 hrs. After leaving the blood to clot for 30 min at room temperature, serum will separate by centrifugation. Then serum was examined for the assessment of biochemical parameters such as triglycerides, TC, LDL, VLDL and HDL, aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin, creatinine, albumin, and blood urea nitrogen. [18]

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

The preliminary qualitative analysis of peteroleum ether and methanol extracts (successive extraction) of *C. wightii* was carried out and their results were given in table (Table 1). Carbohydrates, glycosides, alkaloids, phytosterols, flavonids, phenolic compounds and tannins were found. Methanolic extract of stem bark showed the presence of all phytochemical except proteins and amino acids, saponins, oils and fats. In the petroleum ether extract carbohydrates, alkaloids, glycosides and phytosterol were present where as protein and amino acids, saponins, oils and fats, flavonoid, phenolic compounds and tannins were found to be absent. The result of present study indicates the presence of Carbohydrates, alkaloids, glycosides, phytosterols, flavonids, phenolic compounds and tannins. Different solvents have various degrees of solubility for different photochemical.

Pharmacological Screening

The effect of methanolic extracts of *Commiphora Wightii* on serum lipid profile levels was showed in Table 2. Treatment with methanolic extracts of *Commiphora Wightii* at the doses of 500mg/kg significantly reduced the serum TC, TG and LDLC levels and increased the serum HDL-C levels when compared to the hyperlipidemic control group. The change in lipid levels in groups of II, III and IV were comparable with group of fenofibrate treated rats. Methanolic extracts of *Commiphora Wightii* reduced the elevated lipid levels more significantly and the result conclude, the study showed that administration of *Commiphora Wightii* at dose level 500mg/kg was effective as hypolipidemic agent. The active ingredient present in plant may recover the disorders in lipid metabolism noted in hyperlipidemic state and further work would be necessary to evaluate the active constituents responsible for the activity and mechanisms of these effects.

Table 1: Phytochemical Screening Results for Petroleum Ether and Methanol Extracts

S. No.	Phytoconstituents	Petroleum Ether	Methanol	Ethyl acetate
1.	**Carbohydrate**	Absent	Present	Present
2.	**Proteins & Amino acids**	Absent	Present	Absent
3.	**Alkaloids**	Present	Present	Present
4.	**Glycosides**	Present	Present	Present
5.	**Saponins**	Absent	Presen	Absent
6.	**Phytosterols**	Present	Present	Absent
7.	**Oils and fats**	Present	Absent	Absent
8.	**Phenolic compounds and tannins**	Absent	Absent	Absent
9.	**Flavonoids**	Absent	Present	Present

Model-I High Fat Diet Induced Hyperlipidemia(Extract)

Table 1:- Effect of alcoholic and methonolic extract of root of *Commiphora Wightii* on serum biochemical parameters in cafeteria fed diet rats

The data obtained were analyzed by one way ANOVA followed by Tukey Multiple Comparisons Test. Each values represent the mean ± SEM; n=6. $^{**}p< 0.01$ $^{*}p< 0.05$, $p< 0.001$

Model-II Triton Induced Hyperlipidemia(Extract)

Sr. No	Groups	Cholesterol (mg %)	TGs (mg %)	HDL (mg %)	LDL (mg %)	VLDL (mg %)
1.	Normal Control (2% CMC)	130.9±0.50	70.05 ±2.33	50.79 ±0.93	62.18 ±0.75	13.01±0.51
2.	High fat cafeteria diet	148.8 ±0.89 a^{***}	96.53 ±2.10 a^{***}	20.43 ±0.77 a^{***}	109.2±0.25 a^{***}	18.10±0.75 a^{***}
3.	Cafeteria diet + Fenofibrate (65mg/kg/p.o.)	115.2 ±0.75 a^{***}, b^{***}	55.99 ±2.25 a^{***}, b^{***}	32.98 ±0.77 a^{***}, b^{***}	84.3±0.13 a^{***}, b^{***}	4.95±0.60 a^{***}, b^{***}
4.	Cafeteria diet + Mtoh extract (250mg/kg/p.o.)	135.8 ±0.54 a^{***}, b^{***}, c^{***}, d^{***}	60.0 ±3.15 b^{***}, c^{***},	34.57 ±1.02 a^{***}, b^{***}	90.0±0.42 a^{***}, b^{***}, c^{***}	13.8±0.55 b^{***}, c^{***}, d^{*}
5.	Cafeteria diet +Mtoh extract (500mg/kg/p.o.)	128.4±1.43 b^{***}, c^{***}	70.81 ±3.07 a^{*}, b^{***}, c^{***}	36.58 ±0.77 a^{***}, b^{***}, c^{***}, d^{*}	87.47±0.32 a^{***}, b^{***} c^{***}	12.36±0.70 a^{***}, b^{***} c^{***}

Table 2:- Effect of alcoholic and Methonolic extract of root of *Commiphora Wightii* on serum biochemical parameters in Triton induced hyperlipidemia in rat

Sr. No	GROUP	Cholesterol (mg %)	TG (mg %)	HDL (mg %)	LDL (mg %)	VLDL (mg %)
	Normal Control (2% CMC)	110.34±1.1	90.15±2.7	45.34±2.2	46.97±0.00	18.03±0.01
2.	Triton Control	152.51 + 4.3 a***	153.24 +4.0 a***	27.65+1.6 a**	94.21±0.06 a***	30.62±0.02 a***
3.	Triton + Fenofibrate (65mg/kg/p.o.)	120.15 ± 2.3 b***	90.44 + 7.5 b***	55.84 +1.1 b***	46.26±0.00 a***,b***	18.05±0.00b ***
4.	Triton + Mtoh extract (250mg/kg/p.o.)	140.26 + 2.1 a***,b***, c**	120.37 +5.4 a***,b*, c***	45.56 + 3.2 c***	69.23±0.00 a***,b***, c***	23.47±0.00 a***,b***, c***
5.	Triton + Mtoh extract (500mg/kg/p.o.)	130.10 + 1.1 a*,b***, d**	110.55 +3.1 a***,b***, d**	50.22 +5.1 b***,d*	60.96±0.01 a***,b***, c***,d***	20.92±0.00 a***,b***, c***,d***

The data obtained were analyzed by one way ANOVA followed by Tukey Multiple Comparisons Test. Each values represent the mean ± SEM; n=6. **p< 0.01*p< 0.05, p< 0.001***

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