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PRELIMINARY PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIHYPERLIPEDEMIC ACTIVITY OF VARIOUS EXTRACTS OF GLYCOSMIS PENTAPHYLLA

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ABSTRACT

Hyperlipidemia is abnormally elevated levels of any or all lipids and/or lipoproteins in the blood. It is the most common form of dyslipidemia (which includes any abnormal lipid levels). Lipids (water-insoluble molecules) are transported in a protein capsule. The size of that capsule, or lipoprotein, determines its density. The lipoprotein density and type of apolipoproteins it contains determines the fate of the particle and its influence on metabolism. Hyperlipidemias are divided into primary and secondary subtypes. Primary hyperlipidemia is usually due to genetic causes (such as a mutation in a receptor protein), while secondary hyperlipidemia arises due to other underlying causes such as diabetes. Lipid and lipoprotein abnormalities are common in the general population, and are regarded as a modifiable risk factor for cardiovascular disease due to their influence on atherosclerosis. In addition, some forms may predispose to acute pancreatitis. The main aim and objective of my present research work was the preliminary phytochemical screening of various extracts of seeds of ***Glycosmis pentaphylla*** and evaluation of antihyperlipedemic activity. The experimental data was displayed that the various extracts such **EE-SGPP and ME-SGPP** were showing the lipid lowering ability in

experimental rats with reference to standard drug lovastatin. The declined serum lipids profile by **EE-SGPP** was found to be **76.2, 111.4, 21.1, 32.5, 18.4 mg/dl (TCH, TGS, HDL – C, LDL – C and VLDL – C)** and **ME-SGPP** was found to **be 72.8, 97.4, 20.4, 29., 21.8 mg/dl dl (TCH, TGS, HDL – C, LDL – C and VLDL – C) etc.**

KEYWORDS: Hyperlipidemia, lipoproteins, diabetes, atherosclerosis, antihyperlipedemic etc.

INTRODUCTION

Glycosmis pentaphylla is a species of flowering plant in the citrus family, Rutaceae, known commonly as orange berry and gin berry. It is cultivated for its edible pink fruits. In temperate zones, it can be cultivated indoors as a houseplant. Glycosmis pentaphilla AKA Orange Berry, Gin Berry, or Rum Berry is a beautiful translucent pink berry that is sweet and tastes like tonic water! It is part of the citrus family. If you are in an area that can grow orange tree outdoor, you can grow this outdoor also. In the warmer climate like Southern California, it flowers and fruit most months in the year. When there are no other fruits growing in the garden, these little precious berries are always there to the rescue.[1]

Fig-1: Glycosmis pentaphylla plant.

Fig-2A: Glycosmis pentaphylla aka Gin Berry Seeds.

Fig-2B: Glycosmis pentaphylla aka Gin Berry Seeds.

Fig-3A: 20-fresh-seeds-of-Orange-Gin-Berry-Glycosmis-pentaphylla.

Fig-3B: Glycosmis pentaphylla seeds.

Botany: Gingging is a shrub growing 1 to 5 meters high. Leaves usually have 3 to 5 pinnately arranged leaflets, though these are sometimes reduced to one or two, all forms being often found on the same plant. Leaflets are oblong-lanceolate to lanceolate, 5 to 18 centimetres long, and 2 to 7 centimetres wide. Flowers are small, white, about 6 millimetres in diameter, borne in axillary, solitary or paired, interrupted, narrow, cymose panicles which

are 5 centimetres long or less. Fruit is fleshy, pink or reddish, rounded, 1 centimetre in diameter, and contains a single nearly spherical seed which is about 4 millimetres in diameter. Mesocarp is fleshy and sweet.[1]

CONSTITUENTS

Experimental studied had shown that the presence of following constituents: carbohydrates, alkaloids, flavonoids, tannins and phenolic acids, glycosmin, a crystalline glucoside, present in traces throughout the plant, its greatest concentration found in the new leaves and buds where it reaches 0.2 per cent; in the mature leaves, it varies from 0.08 to 0.1 per cent. Leaves also yield tannin, a phlobaphene, traces of salicin, and about 2.1 per cent of sugars (reducing and non-reducing). From the leaves, study isolated glycolone, a quinolone alkaloid.[2] From the root bark, study isolated carbazole and 3-methylcarbazole.[3] Stem extract yielded a new naphthoquine, glycoquinone, and a new acridone alkaloid, glycocitrine-III along with 12 known compounds.[4]

Properties: Glycosmin is slightly bitter. Considered astringent, vermifuge, anti-inflammatory and expectorant.

Parts used: Stems, roots, bark and leaves.

Uses: Folkloric- Bitter juice of leaves used for fevers, liver complaints and intestinal worms, especially in children. Stems and roots of plant used on ulcers. Paste of leaves, with a bit of ginger, applied to eczema and other skin diseases; also, applied over the navel for worms and other bowel disorders. Infusion of leaves given to women after delivery to induce appetite. Wood is used for snake bites. Used for cough, jaundice, inflammation, rheumatism and anaemia. In Bangladesh, used to reduce blood sugar and to relieve pain. In traditional Indian medicine, used for jaundice and other liver afflictions.

Others: Toothbrush- In eastern Bengal stems used as toothbrushes for its fibrous nature and slightly astringent and bitter quality.

PHARMACOLOGY

1. Anthelmintic: Study showed extracts from G pentaphylla roots showed potent anthelmintic activity on the earthworm, Pheretima posthuma, the methanolic extract with greater activity than the chloroform extract.[5]

2. Hepatoprotective: Study evaluating the hepatoprotective activity of plant materials on Swiss albino rats with liver damage induced by CCl_4 showed G. pentaphylla, B orellana, C caja, and C equisetifolia exhibited moderate dose-dependent protective effect evidenced by lowering of serum enzymes and supported by histopathological studies of liver tissue.[6]

3. Antibacterial: Study of the methanol extracts of seven medicinal plants, including Glycosmis pentaphylla, showed moderate activity against all the tested organisms.[7]

4. Sulfur-Containing Amides / Cytotoxicity: Chloroform extracts of G citrifolia and G elongata yielded a triterpene, four sulfur-containing amides (E-dambullin, Z-dambullin, E-methyldambullin and Z-methyldambulin) and two alkaloids (skimmianine and arborinine). The amides were strongly cytotoxic against a T-lymphoblastic leukemia cell line.[8]

5. Antioxidant: Study showed the extract of leaves of G. pentaphylla and Bauhinia variegata inhibited free radical scavenging activity. The effect was attributed to flavonoids, phenolics and other phytochemical constituents.[9]

6. Antipyretic: Study of ethanolic extracts of B. variegata and Glycosmis pentaphylla exhibited significant antipyretic activities in Brewer's yeast induced pyrexia in rats. Activity was attributed to inhibition of prostaglandin synthesis in the hypothalamus.[10]

7. Antidiabetic / Analgesic: Study evaluated an ethanolic extract for antihyperglycemic and analgesic effects in Swiss albino male mice. Results showed an anti hyperglycemic effect with blood sugar reduction more significant at 120 minutes, comparable to that induced by metformin. The extract also exhibited an analgesic effect comparable to diclofenac sodium.[11]

8. Apoptosis Inducing Effect / Hepatocellular Carcinoma Cell Line: Study evaluated the in vitro anticancer and apoptosis inducing activity of G. pentaphylla in hepatocellular carcinoma cell line, Hep3 B. Results showed concentration and time dependent induction of apoptosis.[12]

9. Anti-Diabetic / Anti-Arthritic / Stem Bark: Study evaluated the antidiabetic and anti-arthritic potential of an ethanolic extract of stem bark of G. pentaphylla. Results showed significant reductions in fasting glucose levels and inflammation observed in diabetic and arthritic animals. Observed increase in insulin levels was attributed to pancreatic ß cell regeneration. There was also significant improvement of hematologic parameters, including ESR.[13]

10. Antinociceptive / Anti-Arthritic / Stem Bark: Study investigated a methanolic extract of leaves for anti-nociceptive activity. The extract produced an antinociceptive effect with

significant inhibition of late phase formalin induced pain and significant decrease in the number of writhing.[14]

11. Antimicrobial / Antioxidant / Cytotoxic / Leaves and Stems: A methanolic extract of stems showed moderate antioxidant activity, probably due to polyphenolic content. Both stem and leaves extracts showed moderate antimicrobial activity. The stems extract showed showed potent cytotoxic activity. Several antitumor alkaloids were identified.[4]

MATERIALS AND METHOD

Drugs and chemicals: The standard drug **Lovastatin** purchased from Local Retail Pharmacy Shop and solvents and other chemicals used for the extraction and phytochemical screening were provided by Institutional Store and were of LR and AR grade.

Experimental animals: White male albino wister rats weighing about 200-250 g were used. They were obtained from the animal house of C.L. Baid Metha College of Pharmacy, Chennai. They were kept under observation for about 7 days before the onset of the experiment to exclude any intercurrent infection, had free access to normal diet and water. The animals were housed in plastic well aerated cages at normal atmospheric temperature (25±5 °C) and normal 12- hour light/dark cycle under hygienic conditions. The experimental protocol was approved by Institutional Animal Ethics Committee **(IAEC) of CPCSEA: IAEC/XXIX/03/2016**.

Methodology for Soxhlet extraction: First dried the seeds of G. Pentaphylla and grind to form a fine powder and placed into the thimble made of stout filter paper and the apparatus is fitted up. The flask containing suitable solvent like ethanol and methanol and is heated on two different water bath or on a heating mental where two **Soxlet apparatus set up.** As the solvent boil, its vapours rise through the side tube up into the water condenser. The condensed liquid drops on the solid in the thimble, dissolves the organic substances present in the powdered material and filters out into the space between the thimble and the glass cylinder. As the level of liquid here rises, the solution flows through the siphon back into the boiling flask. The solvent is once again vaporized, leaving behind the extracted substance in the flask. In this way a continuous stream of pure solvent drops on the solid material, extract the soluble substance and returns to the flask. At the end of the operation the solvent in the boiling flask is distilled off, leaving the organic substance behind.[16] Afterwards the ethanolic and methanolic extracts are transferred in a clean and dried beaker separately and is concentrated by placing on a water bath and cool and then **ethanolic and methanolic**

extracts of seeds of G. Pentaphylla (EE-SGPP, ME-SGPP) are obtained and keep all these extracts in a freeze. From this concentrated extract the preliminary phytochemical screening has to be carried out.

Phytochemical screening: Preliminary phytochemical screening of **EE-SGPP, ME-SGPP** have shown the presence of diverse bioactive molecules such as: carbohydrates, proteins and aminoacids, polyphenols, phytosterols and alkaloids which are confirmed by their specific qualitative cofirmatory chemical tests.[17-20]

Protocol for the study of acute oral toxicity of EE-SGPP and ME-SGPP

In the present study the acute oral toxicity of the **EE-SGPP and ME-SGPP** was performed by acute toxic class method. In this method the toxicity of the extract was planned to test using step wise procedure, each step using three Wister rats. The rats were fasted prior to dosing (food but not water should be withheld) for three to four hrs. Following the period of fasting the animals were weighed and the extract was administered orally at a dose of 2000 mg/Kg b. w. Animals were observed individually after dosing at least once during the first 30 min; periodically the surveillance was carried out for the first 24 hrs with special attention given during the first 4 hrs and daily thereafter, for a total of 14 days.[21]

Screening methodology for antihyperlipidemic activity[22, 23]

A. Experimental Design: Animal: Wister rats, **Sex:** Either Sex, **Weight:** 150-170 gm

On the day of the experiment, the animals were divided randomly into six groups of three animals each.

Group I: Normal (1% Tween 80; 10 ml/kg, p. o)

Group II: Control (Cholesterol (25 mg/kg/day) in oil

Group III: **Test** (**EE-SGPP**; 100 mg/kg in 1% Tween 80) along with cholesterol in oil

Group IV: Test (ME-SGPP; 100 mg/kg in 1% Tween 80) along with cholesterol in oil

Group V: Standard (Lovastatin , 10 mg/kg/day) along with cholesterol in oil

Cholesterol in oil was given by oral route at 10 am and Test drugs or **Lovastatin** was given by oral route at 3 pm daily, to respective groups, for a period of 10 days. The normal control group was treated with vehicle instead of drugs. At the end of the experimental study, animals were fasted for 12 hr and blood was collected by retro-orbital puncture and serum TC, LDL-C, VLDL-C, TAG were determined using Analyser.

RESULTS AND DISCUSSION

Acute oral toxicity study

(i) Acute oral toxicity studies were performed according to the OECD guideline 423 method.

(ii) This method has been designed to evaluate the substance at the fixed doses and provide information both for hazard assessment and substance to be ranked for hazard classification purposes.

(iii) The **extract** was administered initially at a dose of 2000 mg/kg b. w and 1% CMC (p .o) and observed 14 days mortality due to acute toxicity.

(iv) Careful observation were made at least thrice a day for the effect on CNS, ANS, motor activity, salivation and other general signs of toxicity were also observed and recorded.

(v) Since no sign of toxicity observed at 2000 mg/kg b. w. to the group of animals, the **LD_{50}** value of the **extract** expected to exceed 2000 mg/kg b. w. and represented as class 5 (2000 mg/kg < LD50 < 2500 mg/kg).

(vi) From the toxicity studies the data revealed that all the synthesized compounds proved to be non toxic at tested dose levels and well tolerated by the experimental animals as there **LD_{50}** cut of values > 2000 mg/kg b. w.

Table 1: for the dose selection by acute toxicity class method (OECD) guide lines 423 of EE-SGPP and ME-SGPP.

Sl. No.	Treatment group	Dose mg/kg	Sign of toxicity	Onset of toxicity	Duration
1.	EE-SGPP	200	No	No	14 days
2.	ME-SGPP	200	No	No	14 days

Evaluation of antihyperlipidemic activity: The experimental data of **table 2** was displayed that the various extracts such **EE-SGPP and ME-SGPP** were showing the lipid lowering ability in experimental rats with reference to standard drug lovastatin. The declined serum lipids profile by **EE-SGPP** was found to be 76.2, 111.4, 21.1, 32.5, 18.4 mg/dl (TCH, TGS, HDL – C, LDL – C and VLDL – C) and **ME-SGPP** was found to be 72.8, 97.4, 20.4, 29., 21.8 mg/dl dl (TCH, TGS, HDL – C, LDL – C and VLDL – C) etc.

Table 2: Effects of Test

Groups	TCH (mg/dl)	TG_S (mg/dl)	HDL – C (mg/dl)	LDL – C (mg/dl)	VLDL – C (mg/dl)
Normal-I	65.7	68.6	22.8	27.4	13.2
Control-II	88.8	125.5	19.3	37.3	24.1
EE-SGPP -III	76.2	111.4	21.1	32.5	18.4

ME-SGPP -IV	72.8	97.4	20.4	29.9	21.8
Lovastatin-V	57.9	70.2	22.3	28.2	14.7

Extracts on lipid profile in rats.

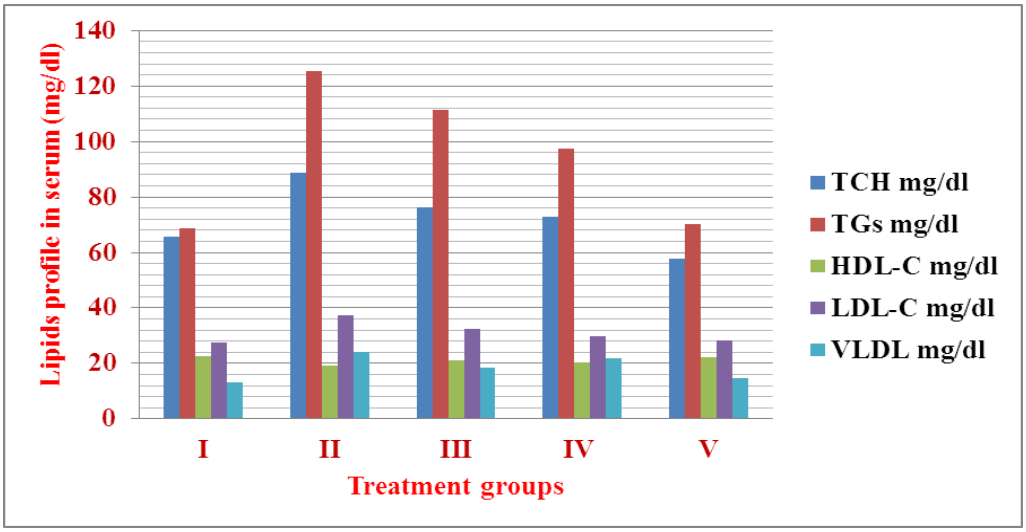

Fig-4: Comparison of serum lipids level in various groups.

CONCLUSION

From the present experimental data here we concluded that the various extracts of seeds of Glycosmis pentaphylla (**EE-SGPP and ME-SGPP**) had the potential ability to lower the plasma lipids profile in experimental rats and this research work given an overview to treat the disease associated with the metabolism of lipids.

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