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Determination of Antitrypsin Activity of Lupeol by Folin Lowery Method

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Abstract:
Lupeol is a compound which found in most of the plants such as Crataeva nurvala. Lupeol was isolated from the stem bark powder of Crataeva nurvala by Soxhlet extraction in 95% ethanol, and then identified by Thin Layer Chromatography, violet colored spot was observed on TLC plates which confirmed the presence of Lupeol. Libbermman Burchard test which is a biochemical test was done for further confirmation of Lupeol in extract. Antitrypsin (Anti-protease) activity was determined by using Folin lowery method which is generally used for protein assay.

Key words*: Crateava nurvala, anti-trypsin*

1. Introduction

Lupeol is a pharmacologically active triterpenoid found in a variety of fruits such as olive, mango, strawberry, grapes and figs, in many vegetables, and in several medicinal plants, including *Crataeva nurvala*, *Aleo Vera* etc. It has several medicinal properties, like anti inflammatory, antiplasmodial, antioxidant (Anandjiwala *et.al*, 2007, Geetha & Varalakshmi 2001). *Crataeva nurvala* Buch Ham (Capparidaceae) is a high-value medicinal tree that grows almost all over India, especially in the semiarid regions. Medicinal usage has been reported in traditional systems of medicine, such as Ayurveda and Unani, wherein the plant is frequently preferred in the treatment of urinary disorders (Bopana and Saxena 2011). Triterpenoids, lupeol and varunol, have been isolated from the root and stem bark of *Crataeva nurvala.* The family Capparidaceae comprises about 45 genera and 700 species of trees, which are distributed mainly in the warmer (tropical) parts of the world. The plants occur mostly in dry seasons. Several shrubby species of Capparis occur in the Mediterranean region, while few genera, such as *Capparis*, *Gynandropsis*, *Cleome*, *Crataeva*, etc are found in Bangladesh (Ghani, 2003). A wide variety of medicinally important compounds, including fraudulent, diosgenin, sitosterol, butulinic acid and betulinaldehyde have been reported from *C. nurvala* (Lakshmi, V. and Chauhan, J.S. 1975). The bark of *C. nurvala* is contraceptive and cytotoxic and is especially useful in urinary disorders, kidney bladder stones, fever, vomiting and gastric irritation (Gagandeep, M. and Kalidhar, S.B. 2006). Active principle, lupeol, is found in the highest concentrations in the stem bark of *Crataeva nurvala*. Although all plant parts, including roots, stem, bark, leaves, and flowers, are of medicinal value. According to the Indian Herbal Pharmacopoeia, the major chemical constituent of the root bark of *Crataeva nurvala* is lupeol. Other major constituents of the root bark of *Crataeva nurvala* include lupeol acetate, a-spinasterol acetate, y-taraxasterol, 3-epilupeol, and b-sitosterol (Varlaxami *et al*, 2001). [(Lupa-21, 20(29) dien, 3-beta-ol) (3b)-Lup-20 (29)-en-3-0l], Lupeol, a triterpene alcohol is a major chemical constituent of root and stem bark of C. *nurvala* (Lakshmi V and Chauhan *et al* 1975). It is an abundant plant triterpene and also found in the seed coat of *Lupinus* (Lupin) seeds, chicle and in the latex of fig and rubber plants. The trunk bark of *Crataeva nurvala* when extracted with alcohol and defatted with light petroleum followed by concentration in vacuum, yields crude lupeol (0.7%). Purified Lupeol could be obtained from this fraction on recrystallization From ethyl acetate and ethanol. Lupeol is a monohydric alcohol, with molecular formula $C_{30}H_{50}O$ (Wal, Ankita *et al*, 2010).

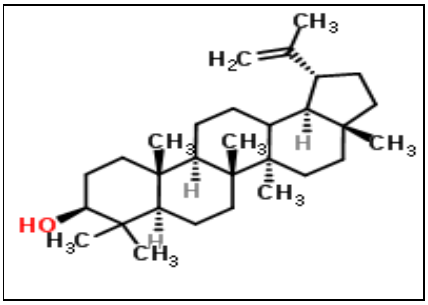

Figure 1: Structure of Lupeol

2. Materials and methods

2.1. To detect the presence of lupeol in stem bark powder of Crataeva nurvala and other plant samples by Libbermman Burchard test

2.1.1. Materials
Stem bark powder of *Crataeva nurvala*, Plant samples such as *Aleo Vera*, Carrot , Carrot roots, Test tubes, Pipettes, soxhleted extract of *Crataeva nurvala*, 95% ethanol, Chloroform, Acetic anhydride, Sulphuric acid

2.2.2. Method
Powdered sample of *Crataeva nurvala* was carefully weighed. For other plant samples such as *Aleo Vera*, Carrot fresh wet tissue, 1g each was carefully weighed and homogenized in 10ml of 95% ethanol using pestle and mortar. The extracts were collected in separate test tube and were tested for the presence of lupeol by Libbermman Burchard test for cholesterol (Deb.A.C, 1992*)*.
First 1ml of extract was taken in separate test tube then 1ml of chloroform was added in each test tube followed by the addition of acetic anhydride and was mixed by swirling, finally 3 drops of sulphuric acid were added. It was incubated for half an hour and color change was observed.

2.2. Soxhlet extraction of stem bark powder of Crataeva nurvala

2.2.1. Materials
Stem bark powder of *Crataeva nurvala.*, Soxhlet apparatus , 95% ethanol, Muslin cloth

2.2.2. Method
Stem bark powder of *Crataeva nurvala* was extracted to yield the secondary metabolite lupeol using Soxhlet apparatus.
Stem bark powder of *Crataeva nurvala* (20g) was carefully weighed and was tied loosely in a piece of muslin cloth. This loosely tied bundle was placed in the sample chamber (thimble) of Soxhlet apparatus. 150ml of 95% ethanol was poured in the lower flask and the assembly was places on a heating mantle. Soxhlet extraction was done for three days by heating at 60°C.
This extract was used in the further tests.

2.3. Identification of lupeol by TLC analysis

2.3.1. Materials
Soxhleted extract of stem bark of *Crataeva nurvala* ,Microscopic slides, Beaker 250ml, Glass plate, Benzene: ethyl acetate (97.5:2.5), Silica gel60 F254.

2.3.2. Method
Solvent system was prepared of Benzene: ethyl acetate in a ratio of 97.5:2.5, TLC plates were prepared by using silica gel60 F254. These plates were dried and incubated at 110^0 C for half an hour to activate them. Ten microliters of soxhleted extract was spotted on the activated plate and were developed in solvent system.

2.4. Determination of anti-trypsin activity of lupeol by Folin Lowery method

2.4.1. Materials
Test tubes, Beakers, Conical flasks, Pipettes, Spectrophotometer, Cuvetts, Folin reagent, Casein solution 0.2mg/ml, 50mM of potassium phosphate buffer pH 7.5 110mM ,Trichloroacetic acid stock solution, Trypsin solution 0.25%, Heat killed trypsin was used as Inactive trypsin

2.5. Preparation of Reagents
Analytical solution was prepared from solution A and solution B, Reagent A: 2% of sodium carbonate solution mixed with 0.1N NaOH solution in 50ml, Reagent B: 1.56% copper sulphate , Analytical reagent was prepared by mixing 2ml of reagent (B) with 100ml reagent (A), Inhibitor was prepared in 70% ethanol (1mg/ml concentration).

2.5.1. Method
Casein solution was added in all test tubes, 0.5 ml in each test tube. Then potassium phosphate buffer was added serially as shown in the table no.1. Inhibitor was added 0.1ml, 0.3ml and 0.5ml in respective test tubes followed by 70% ethanol. Inactive enzyme was added 1ml in one set (set1) as shown in table no.1. Active 1ml Trypsin was added in remaining test tubes and was incubated for an hour at room temperature. 5ml of chilled TCA was added in all test tubes to stop the enzymatic reaction and was mixed well. This was further centrifuged to get clear supernatant at 600 rpm. 1ml of supernatant was transferred to another test tube and 1ml of water was added to make a volume upto 2ml. 5ml Sodium carbonate solution was added in all test tubes and incubated for 10min. After incubation 1ml of Folin ciocalteiu reagent was added in all test tubes and incubated for 30 min. Absorbance was taken at 750nm.

- Analysis of lupeol in Chemo-informatics tools

3. Result and Discussion

Identification of lupeol in crude extract was done by Libbermman Burchard test (Campbell *et al.* 2005) which shows the color change in the test solution. Lupeol gives Libbermman Burchard test positive because the structure of lupeol is similar to that of cholesterol molecule, The color is due to the hydroxyl group (-OH) of Lupeol reacting with the reagents and increasing the conjugation of the unsaturation in the adjacent fused ring. Since this test uses acetic anhydride and sulfuric acid as reagents.
Thin layer chromatography also shown violet colored spot on TLC plates in solvent system of Benzene: ethyl acetate in 97.5: 2.5 this was confirmed by comparing results with earlier work done on TLC studies of Lupeol.

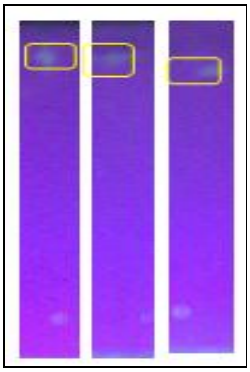

Figure 2: TLC plates showing violet colored spot of lupeol

Folin lowery method was used for the antitrypsin activity determination of Lupeol by using soxhleted extract and it was found that at 1mg/ml concentration Lupeol has shown 100% inhibitory activity for Trypsin which is used in enzyme assay.

4. Observation and Results

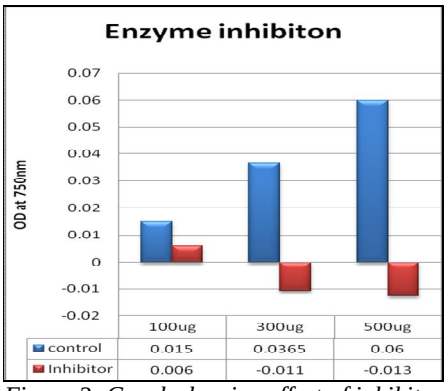

Figure 3: Graph showing effect of inhibitor

Serial number	Concentration of inhibitor(mg/ml)	Concentration of inhibitor (µg/ml)	Inhibition in percentage(%)
1	0.1mg/ml	100µg/ml	60%
2	0.3mg/ml	300µg/ml	100%
3	05mg/ml	500µg/ml	100%

Table 1: Percent inhibition by Inhibitor

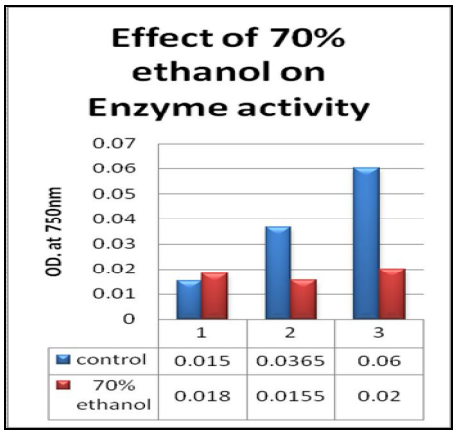

Figure 4: Graph showing effect of 70% ethanol on normal enzymatic reaction.

Sr.no.	70% ethanol in ml	Percent inhibitory effect on Trypsin activity
1	0.1ml	0%
2	0.3ml	57%
3	0.5ml	66%

Table 2: Percent inhibition by 70% ethanol

5. Conclusion

As Lupeol is a triterpene compound and having structural similarities with cholesterol, Lupeol has given Libbermman Burchard positive test, in this test –OH group of Lupeol which is similar to cholesterol make it able to bind with other reagents in the reaction mixture such as acetic anhydride.

Results obtained from inhibitory assay it can be concluded that there decrease in the enzyme activity was upto 60%, 100%, 100% when Lupeol was added as inhibitor. After comparison between the graph of inhibitor and 70% ethanol it can be concluded that Lupeol is found to be more effective then only by the use of 70% ethanol.

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