PHYTOCHEMICAL STUDY ON THE LEAVES OF ALSTONIA SCHOLARIS AND THEIR EFFECTS ON PATHOGENIC ORGANISMS

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ABSTRACT: Many diseases are being spread in the world by microorganisms. This necessitates the development of cost effective and easily available antimicrobial medicines. Plants have generally been source of alkaloids, steroids, terpenoids, carbohydrates, amino acids, vitamins, glycosides and various pigments. The chemical exploitation of varieties of indigenous plants is therefore likely to offer a cost effective treatment for many diseases leading to the development of the nation and welfare of the society. The present communication reports that hydrocarbons, triterpenes and phytosterols present in Alstonia scholaris, are responsible for its medicinal value.

INTRODUCTION

Alstonia scholaris R. Br. (Apocynaceae) which is commonly known as Datyuni and Chatium in Hindi, Devil tree in English and Saptaparna in Sanskrit is a small evergreen tree with bitter milky juice. The milky juice of the tree is applied to ulcers. The bark is acrid, bitter, appetizer, laxative, anthelmintic, a reputed remedy for malaria and also stated to be efficacious in heart diseases, asthma, leucoderma, tumours and very good for treatment of chronic ulcers, chronic diarrhoea. It is used as an astringent, tonic and febrifuge1-2. Alcoholic extract of stem bark showed anticancer activity in HS1 human sarcoma in the embryonated egg3. It also finds its use as antidysentric and antiseptic4.

In view of its medicinal importance, the compounds isolated from the plant and their acetyl derivatives were screened against Staphylococcus aureus, Staphylococcus albus, Bacillus subtilis (gram-positive), Escherichia coli, Klebsiella, Shigella dysenteriae, Proteus vulgaris and Pseudomonas pyocyanea (gram-negative) in vitro for the first time and the results are communicated.

EXPERIMENTAL

Air dried and powdered leaves of the plant collected from the surroundings of Aligrah district were extracted in boiling petroleum ether (60 – 80°C). Chromatographic separation of this extract over a column of silica gel by eluting it with petroleum and petroleum ether with increasing amount of benzene gave following fractions:

n-alkanes (C23 – C33), esters (C40 – C52), alkanols (C24-C30), triterpenes, α-amyrin, lupeol, and sterols (Cholesterol, Campesterol, Stigmasterol, β-sitosterol and stigmast – 7 enol).
Triterpenes and sterols were converted into their acetates by acetic anhydride and pyridine. Two major products, stigosterylacetate and stigmasteryl acetate, were separated from the mixtures of sterylacetate by argentation (Silica gel – 20% Ag NO₃). Column chromatography by eluting the column with light petroleum – benzene (19:1 v/v and 9:1 v/v) respectively and crystallizing from methanol gave fine crystals of β-sitosterol acetate (M.P. 123°C) and stigmasterol acetate (M.P. 144°C).

These steryl acetates along with n-alkanes, esters, alkanols, triterpenses and sterols from the leaves of *Alstonia scholaris* were separately screened *in vitro* by marble cup method⁵ against *S.aureus*, *S. albus*, *B.Subtilis* (gram-positive) and *E-coli*, *Klebsiella*, *Shigella dysentriae*, *Proteus vulgaris* and *Pseudomonas pyocyanea* (gram-negative).

RESULTS AND DISCUSSION

The antibacterial activity shown by different compounds isolated from the *Alstonic* species indicates that the long chain hydrocarbons are active against both the gram-positive and gram-negative bacteria. While esters and alcohols are totally inactive against all types of the selected bacteria, it has also been observed that α-amyрин and lupeol are more active against all the selected bacteria except *Bacillus subtilis*, *Escherichia coli*. Sterols as compared to aliphatic long chain hydrocarbons are either more or equally active against all selected gram-negative bacteria. Thus the introduction of acetyl group in α-amyрин, lupeol and sterols either decreases the activity as in *Shigella aureus*, *Staphylococcus albus*, *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas pyocyanea* or has no effect as in case of *Klebsiella* and *Shigella dysentriae*.

**TABLE – 1**

| COMPOUND   | Shigella 1mg/ml | Auerus 0.5mg/ml | Staphylococcus 1mg/ml | Albus 0.5mg/ml | Bacillus 1mg/ml | Subtilis 0.5mg/ml |
|------------|----------------|----------------|----------------------|---------------|----------------|------------------|
| Alkanes    | ++             | ++             | ++                   | +             | ++             | +                |
| Esters     | -              | -              | -                    | -             | -              | -                |
| Alkanols   | -              | -              | -                    | -             | -              | -                |
| α-amyрин   | ++             | +              | ++                   | ++            | +              | +                |
| Lupeol     | ++             | -              | ++                   | ++            | +              | +                |
| Sterol     | +++            | +              | ++                   | +             | -              | -                |
| α-amyrinacetate | +          | +              | +                    | +             | +              | -                |
| Lupeol acetate | +          | +              | +                    | +             | +              | -                |
| COMPOUND           | *Eschrichiacoli* | *Klebsiella* | *Shigella dysentriae* | *Proteus vulgaris* | *Pseudomonas pyocyanaea* |
|--------------------|------------------|--------------|-----------------------|--------------------|-------------------------|
|                    | 1 mg/ml          | 0.5 mg/ml    | 1 mg/ml               | 0.5 mg/ml          | 1 mg/ml                 |
| Alkanes            | +++              | ++           | ++                    | +                  | +++                     |
| Alkanols           | -                | -            | -                     | -                  | -                       |
| Sterol             | +++              | ++           | ++                    | +                  | -                       |
| Steryl acetate     | ++               | +            | ++                    | -                  | +                       |
| Sitosteryl acetate | +++              | +            | -                     | -                  | +                       |
| Stigmasteryl acetate | ++             | +            | -                     | +                  | +                       |
| Esters             | -                | -            | -                     | -                  | -                       |
| α-amyrin           | +                | +            | ++                    | ++                 | +++                     |
| Lupeol             | +                | +            | -                     | +                  | ++                     |
| α-amyrinacetate    | +                | -            | -                     | -                  | +                       |
| Lupeol acetate     | +                | -            | -                     | -                  | +                       |

Increase in diameter of zone of inhibition: (+) = 0.2 cm, (+++) = 0.6 cm, (++) = 0.4 cm, (-) = inactive
When these compounds were tested at half concentration i.e. 0.5 mg of the compound dissolved in 1 c.c. benzene and keeping four drops of each test solution of compound into holes of medium in Petri dishes containing agar-agar prepared in liquid media having peptone beaf extract, yeast extract, NaCl and glucose at pH 7.4 – 7.6, their activity showed relative decrease in most cases but remained similar in few cases. Thus it may be concluded that hydrocarbons, triterpenes and sterols are active constituents of these plants against the selected micro-organisms. (Table1 & 2).

The interesting observations reported earlier that β-sitosterol possess anti-inflammatory activity similar to hydrocortisone and oxyphenbutazone and antipyretic activity similar to acetyl salicylic acid also suggests that the presence of hydrocarbons, triterpenes and phytosterols in these plants might be responsible for their medicinal value. Thus the selected plant may prove itself useful and might be used for the treatment of disease like dysentery, asthma and urinary tract infections.

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REFERENCES

1. Kirtikar, K.R.; Basu, B.D.; Indian Medicinal Plants, Vol. II, P.565, (1975).

2. Singh V.K., Khan A.M., Medicinal Plants and Folk-lores, Today, and Tomorrow’s Printers, New Delhi India, P.5, (1990)

3. Medicinal Plants of India, I.C.M.R., p.48, (1975).

4. Chopra, R.N. Nayar, S.L., Chopra, I.C., Indigenous Drugs of India, C.S.I.R., p. 595 – 609.

5. Ghedira, K.Z., Richard B.M., Le Men-Olivier, Goh S.H., Phytochemistry 27 (12). P 3955-3962, (1990).