Antibacterial Activity of U. indica Extract Obtained by Maceration Method

R. Manigandan[1], P. Priya[1], R. Suresh[1], K. Giribabu[1], S. Munusamy[1], S. Praveen kumar[1], S. Muthamizh[1], A. Bijul Lakshman[2], and V. Narayanan[1*]
[1]*Department of Inorganic Chemistry, University of Madras, Guindy Campus, Chennai 600 025.*
[2]*Selvam college of technology, Pappinayakkanpatti, Namakkal, Tamilnadu.*
** vnnara@yahoo.co.in*

Abstract

Pulverized plant material of *Urginea indica (U. indica)* was cold extracted with various solvents such as hexane, benzene, ethyl acetate and ethanol by maceration method. Identification of volatile and semivolatile organic compounds in the extract was analyzed using GC-MS technique. Crude extract was subjected to phytochemical analysis & anti-bacterial study. The disc diffusion method was used to evaluate the anti-bacterial activity of *U. indica* extract. *Urginea indica* (Indian squill) reveals, better efficacy against pathogenic bacteria and this might be due to presence of bioactive constituents as related to the other existing wild Onion varieties. Finally, it has been found that the ethyl acetate extract of *Urginea indica* has high anti-bacterial activity compared with other protic, aprotic and non-polar solvents.

Keywords- *Urginea indica*; Maceration method; Cold extraction; Antibacterial activity;

I. INTRODUCTION

The presence of antibacterial substances in the medicinal plants is well established. Plants have provided a source of motivation for novel drug compounds as plants derived medicines have made substantial contribution towards human health [1]. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine, a natural blueprint for the development of a drug [2]. Urginea is commonly called as Indian squill finds its application in pharmaceuticals as well as in agriculture. Because of the popularity of the digitalis glycoside squill components, its use is restricted in United States as cardioactive agents. A team of researchers were still working on developing a cytological database on Urginea indica [3]. Occurrence and its importance in evolutionary diversification of species were reported in diverse species of angiosperms. Meiotic irregularities & variations in the chromosomal behaviour indicate that the populations could eventually be treated as chromosome races [4].

In recent years, the interest for the study of the organic compounds from plants and their activity has increased [5]. The combination of an ideal separation technique (GC) with the best identification technique (MS) made GC–MS an ideal technique for qualitative and quantitative analysis for volatile and semi-volatile compounds. The aim of the present study was to develop a rapid method for the quantitative determination of organic compounds in plant and to confirm the phytochemicals present in the plant extracts. In the present study, we have isolated and studied the phytochemical and anti-bacterial activity of *U. indica.*

II. EXPERIMENTAL

A. Materials and Method

Sliced plant (*Urginea indica)* was collected from Tamilnadu, India. Muller-Hinton agar, Tween 20, hexane, benzene, ethyl acetate and ethanol were of analytical grade and used as obtained. Bacterial cultures of Escherichia coli were obtained from the Microbiology laboratory.

B. Preparation of Extract

Extraction of dried and powdered plant (4.5 kg) of *U. indica* was done by cold extraction. The dried *Urginea indica* and the powder was packed in the thimble of a soxhelt apparatus and extracted with 1000ml at 100c for 48 hrs. u.indica bulbs are prepared by simple maceration in an air tight container fine powder material are merged in the respective solvent and the homogenates are kept for 2 weeks at room temperature. The air dried and pulverized plant material (141.86gm) was cold extracted with different solvents (hexane, benzene, ethyl acetate and ethanol). And after that the fractions were evaporated by roto-dryer at low temperature (40−50°C) to dryness. Crude extract was subjected to phytochemical Analysis & anti-bacterial activity study.

C. Phytochemical Screening

A small portion of the dry extracts was screened for the presence of chemical constituents like alkaloids, carbohydrates, glycosides, polyphenols, saponins, phytosterols, flavanoids, proteins and terpenoids. All extracts were dissolved in HPLC grade methanol and subjected to GC and MS JEOL GC mate equipped with secondary electron multiplier. JEOL GCMATE II GC-MS (Agilent Technologies 6890N Network GC system for gas chromatography). The column (HP5) was fused silica 50 m x 0.25 mm I.D. Analysis conditions were 20 min at 100°C, 3 min at 235°C for column temperature, 240°C for injector temperature, helium was the carrier gas and split ratio was 5:4. The sample (1 µl) was evaporated in a split less injector at 300°C. Run time was 22 min (13). The compounds were identified by gas chromatography coupled with mass spectrometry.

D. Antimicrobial Activity

The antimicrobial activity was evaluated by measuring the diameter of inhibition zone. Mueller Hinton agar, with 0·5% (v/v) Tween-20 but no oil, was used as a positive growth control. Inoculated plates were incubated at 35°C for 48h. Minimum inhibitory concentrations (MICs) were determined after 24h for the bacteria and after 48h. The MICs were determined as the lowest concentration of oil inhibiting the visible growth of each organism on the agar plate.

III. RESULT AND DISCUSSION

Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers use primarily water as the solvent but we found in this study the plant extracts by ethyl acetate provided more consistent antimicrobial activity compared to those extracted by other solvents.

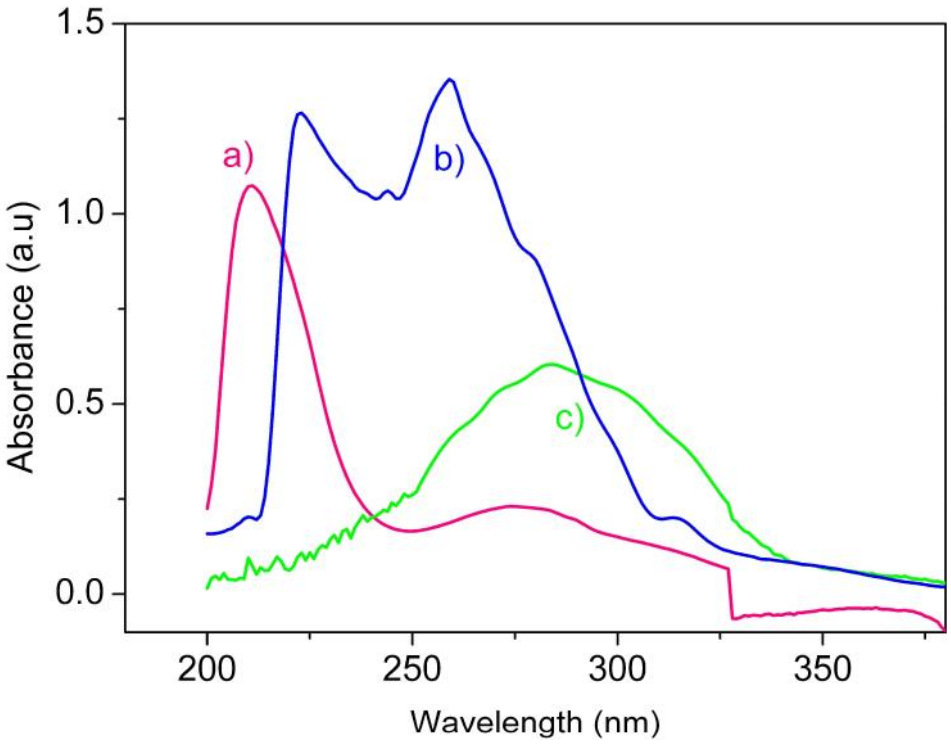

Fig. 1 UV-Vis absorption spectra of *U. indica* extracted using a) Ethanol, b) Hexane and c)ethyl acetate

The UV absorption spectra obtained from *U. indica* extracts from 180 to 380 nm can be seen in Figure 1. Fig 1a show two broad peaks at 230 and 280 nm, characteristic of polyphenols and other absorbing components in the indica extracts. Extraction of phenolic acids and flavonols was carried out using 80% methanol in water based on previous optimization study on extraction of phenolic acids from onion.

The identification of the different phenolic phytochemicals (2-Butanol (Dimethylamino)-2 methyl benzoate, Trans-2,4-Dimethyl Dioxide 3-N-hexylthiane Dioxide, 2-Azido-2,4,4,6,6,8,8-heptamethyl nonane, Ethyl 9 ,12 hexadeca dienoate, Hexane-1,1-diethoxy, 2H-2,4A methanonaphthalene 1,3,4,5,6,7 hexahydro-1,1,5,5-trimethyl,(2S), 2-Thiopheneacetic acid,undec-2-ethyl ester, Dihexadecyl phosphate, Alpha-d-xylofuronoside, methyl 2-o-methyl, 1,2 Benzedicarboxyl acid, Bis(1 methyl) propyl ester, Squalene, Cyclobuta carbonylic acid, pent-2-Uranyl ester, 1,R,2c,3T-Tetra methyl,cyclo hexane, Dicyclohexyl, methyl phosphonate, Ethyl,15 methyl

hexadecanoate, Ethyl 9,12 hexaolecadinoate) was achieved by comparison of UV–vis and GC-MS spectral analysis data given in Figure 1 & 2.

S.NO	PHYTO-CHEMICALS
1	2-Butanol,1(Dimethylamino)-2 methyl,benzoate.
2	Trans-2,4-Dimethyl,Dioxide.
3	3-N-hexylthiane,Dioxide.
4	2-Azido-2,4,4,6,6,8,8-heptamethyl nonane.
5	Ethyl.9.12hexadeca dienoate.
6	Hexane-1,1-diethoxy.
7	2H-2,4A methanonaphthalene,1,3,4,5,6,7 hexahydro-1,1,5,5-trimethyl,(2S).
8	2-Thiopheneacetic acid,undec-2-ethyl ester.
9	Dihexadecyl phosphate.
10	Alpha-d-xylofuronoside,methyl 2-o-methyl.
11	1,2,Benzedicarboxyl acid, Bis(1 methyl) propyl ester
12	Squalene.
13	Cyclobuta carbonylic acid, pent-2-Uranyl ester.
14	1,R,2c,3T-Tetra methyl,cyclo hexane.
15	Dicyclohexyl, methyl phosphonate.
16	Ethyl,15,methyl,hexadecanoate.
17	Ethyl 9,12 hexaolecadinoate.

Table 1. The Important Phyto-chemicals Compounds and the attached basic principle components responsibie for bio-activity.

The obtained extracts were subjected to preliminary phytochemical screening for various compounds and the results showed that they contain mostly alkaloids, phenols, saponins, phytosterols and terpenoids. Preliminary phytochemical analysis revealed the presence of alkaloids and flavonoids, though the latter was in lesser amount. The other secondary metabolites like tannins, saponins, steroids, cardiac glycosides, etc. were present in trace amounts in some of the plants. Diversity of medicinal plants and herbs containing various phytochemicals with biological activity can be of valuable therapeutic key. Gc-Ms Chromatograms (Fig. 2) of leaf extract (*U. indica)* with different solvents shows the peak area separations. Different phytochemicals found to have a broad range of activities, which may help in protection against chronic diseases. The details of the identified phytoconstitutents were given in the Table 1.

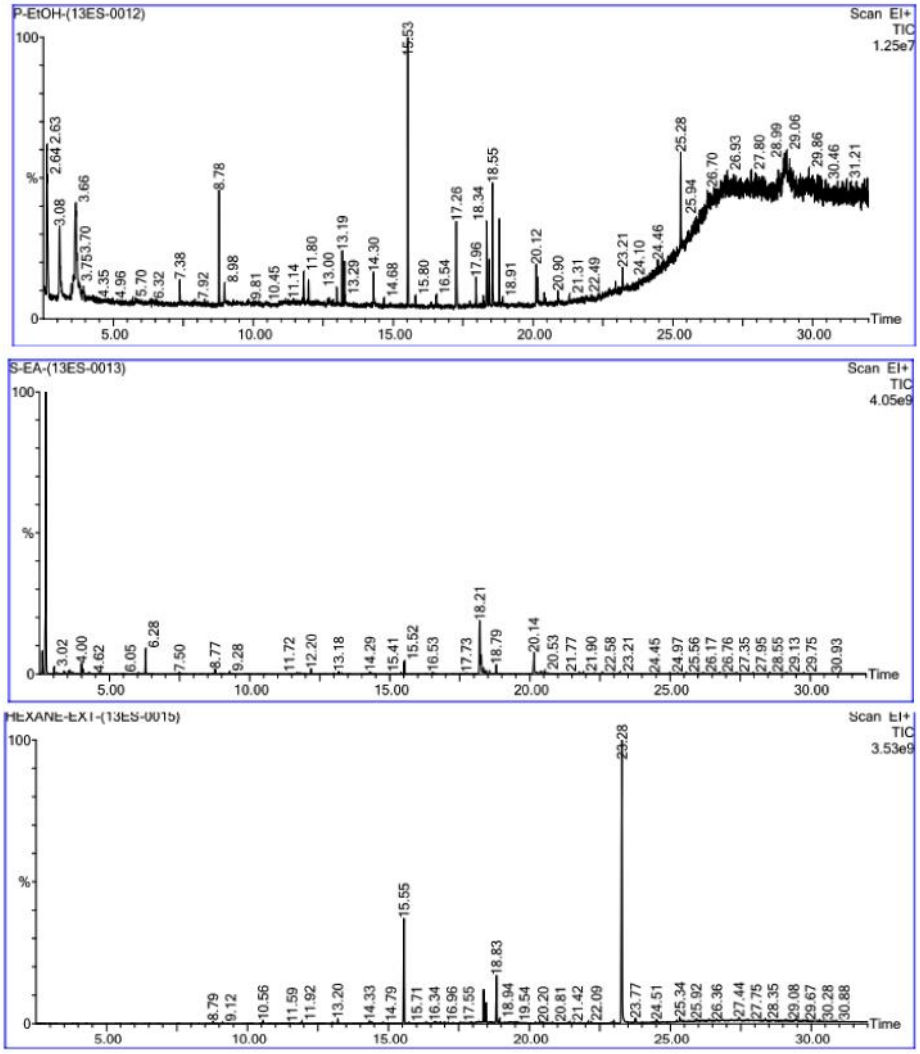

Fig 2. Gc-Ms Chromatograms of leaf extract (*U. indica)* with different solvents.

There is an increasing interest in the phytochemical compounds, which could be relevant to their nutritional incidence and their role in health and disease [6]. The results of antibacterial activity of all the 4 different extracts against the investigated bacterial strains are shown in Figure 3. It is surprising that there are differences in the antimicrobial effects of plant group extracted with different solvent, due to phytochemical properties and differences among species. Polar solvent extracts (ethanol and ethyl acetate) showed the better antibacterial activity Compared with the non-polar solvent extracts (benzene and hexane). None of the non-polar extracts produced zones of inhibition in the diffusion analysis. This might have resulted from the lack of solubility of the active constituents while ethyl acetate extract showed some degree of antibacterial activity against the most resistant bacteria Escherichia coli (E. coli). Further trials using solvents of various polarities will explore the effects of solvent composition on extract efficacy. This trend might be similar in E. aerogenes, other Gram negative bacteria. Various workers have already shown that Gram positive bacteria are more susceptible towards plants extracts as compared to Gram negative bacteria [7]. In addition, microorganisms show variable sensitivity to chemical substances related to different resistance levels between strains. In this study the leaf extract by ethyl acetate provided more reliable antibacterial activity compared to those extracted by other solvents (benzene, hexane, and ethanol).

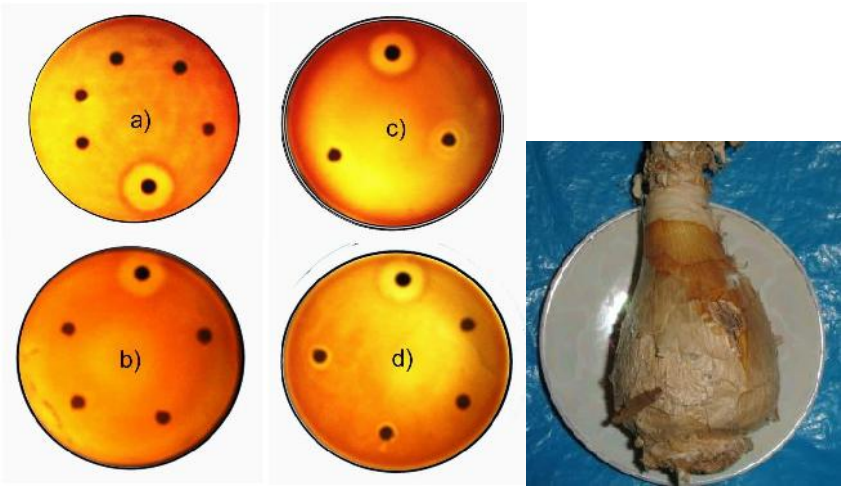

Fig 3. Antimicrobial activity of *U. indica* extract. a) benzene b) hexane c) ethyl acetate d) ethanol (Right) Dried *U. indica* plant.

IV. CONCLUSION

Pulverized plant material of *U. indica* was cold extracted with various solvents such as hexane, benzene, ethyl acetate and ethanol by maceration method. Finally, it has been found that the ethyl acetate extract of *U. indica* has high anti-bacterial activity compared with other protic and non-polar solvents. Amongst the species investigated, ethyl acetate extract of *U. indica* showed the most remarkable activity aprotic solubility due to the presence of therapeutically potent compound. This plant can be further subjected to isolation of the therapeutic antimicrobials and carry out further pharmacological evaluation.

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