University Grants Commission

Minor Research Project

Executive Summary of the report

PROJECT NO.: 47-2044/11 (WRO)

PROJECT TITAL: Determination of Bioactivity of some indigenous plant From Maharashtra.

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Malaria is a serious public health problem in many countries. Vector born diseases, such as malaria, yellow and dengue fevers still cause thousands of deaths per year. The *Aedes aegypti* mosquito is the primary carrier for viruses that cause Dengue Fever and Yellow Fever often called the "Yellow Fever Mosquito,". *Culex quinquefasciatus* (Disease monger, Southern hous mosquito) is dominating vector of lymphatic filariasis, a disease caused by threadlike parasitic worms. The world health organization estimates that approximately 80 % of the world's population relies primarily on traditional medicines as source for their primary health care. Many efforts have been focused on plant extracts, as phytochemicals are potent sources of commercial mosquito control agents or as lead compounds.

- *Himalayan doop,* local name, has medicinal uses in Ayurveda and Tibetan medicine. The part of tree used as Mosquito repellent by local people in manali region. *Sterculia guttata, Sterculia foetida* and *Pterospermum acerifolium* are medicinal important plant which belongs to Sterculiaceae family. *Sterculia guttata* is medicinal important plant which belongs to Sterculiaceae family. *Sterculia foetida* L. is a tropical plant belonging to the Sterculiaceae family which is also called as 'Java-Olive'. The leaves of this plant are used as herbal medicine as aperients, diuretic and as insect repellent.

In search of the medicinal plants in Maharastra, *Sterculia guttata* Roxb., *Sterculia Foetida , Pterospermum acerifolium* from (Sterculiaceae) family, Ruta chalepensis L, Atalantia rasemosa from (Rutaceae) family and other than Maharashtra region one of the Plant commonly known as Himalayan doop collected from Manali region, Himachal Pradesh were selected for investigation. Out of these six plants, after literature survey and

authentication, three from strculiaceae family and one Himalayan doop were screen for preliminary investigation and then for biological activity.

The present work is divided into THREE sections.

In SECTION–I, Phytochemical investigation of *Sterculia guttata* Roxb., *Sterculia Foetida* , *Pterospermum acerifolium* from (Sterculiaceae) family and Himalayan doop of these four medicinal plants has been carried out. In preliminary screening experiments, detection of sugar, alkaloids, steroids, starch, and tannins etc. has been carried out by Phyto-chemical test. Detection of secondary metabolite by Thin Layer Chromatography from different solvent extracts by spraying reagents was also carried out.

In the SECTION-II, Polar, non-polar and semi-polar soxhlet crude extracts of *Sterculia guttata* Roxb., *Sterculia Foetida* , *Pterospermum acerifolium* from (Sterculiaceae) family and Himalayan doop were screen for their larvicidal activity.

In SECTION-III, Isolation and purification of secondary metabolite from Potent Extracts were carried out by column chromatography technique.

Non-Polar, Semipolar and Poar soxhlet crude soxhlet extracts of *Himalayan doop*, *Sterculia guttata* Roxb., *Sterculia Foetida*, *Pterospermum acerifolium* from (Sterculiaceae) family were screen for their larvicidal activity against *Aedes aegypti* and *Culex quinquefasciatus*.

***Himalayan Doop* (A):** Hexane, chloroform followed by Methanol extract of *Himalayan Doop* (100 g) were obtained using soxhlet extractor. Solvents were removed under reduced pressure and dried to get n-Hexane $\mathbf{A_1}$, 12.43g (12.43%), Chloroform $\mathbf{A_2}$, 2.25g (2.25%) and Methanol $\mathbf{A_3}$, 5.67g (5.67%) extracts.

***Sterculia guttata* (B):** Hexane, chloroform followed by Methanol extract of *Sterculia guttata* (100 g) were obtained using soxhlet extractor. Solvents were removed under reduced pressure and dried to get n-Hexane $\mathbf{B_1}$, 3.15g (3.15%), Chloroform $\mathbf{B_2}$, 2.29g (2.29%) and Methanol $\mathbf{B_3}$, 4.77g (4.77%) extracts.

***Sterculia foetida* (C):** Hexane, chloroform followed by Methanol extract of *Sterculia foetida* (100 g) were obtained using soxhlet extractor. Solvents were removed under reduced pressure and dried to get n-Hexane $\mathbf{C_1}$, 2.88g (2.88%), Chloroform $\mathbf{C_2}$, 1.90g (1.90%) and Methanol $\mathbf{C_3}$, 12.16g (12.16%) extracts.

***Pterospermum acerifolium* (D):** Hexane, chloroform followed by Methanol extracts of

Pterospermum acerifolium, (100 g) were obtained using soxhlet extractor. Solvents were removed under reduced pressure and dried to get n-Hexane **D_1,** 5.33g (5.33%), Chloroform **D_2**, 1.99g (1.99%) and Methanol **D_3,** 6.03g (6.03%) extracts.

Direct Ethanol Extract of *Himalayan Doop* (100g), *Sterculia guttata* (100g), *Sterculia foetida* (100g) and *Pterospermum acerifolium* (100g) were obtained using soxhlet extractor. Solvents were removed under reduced pressure and dried to gave **A_4**=17.25g (17.25%), **B_4**= 34.15g (34.15%), **C4**=5.1g (10.2%), and **D4**=34.25g (34.25%).

- Out of all the extracts, **A_1** and **A_2** exhibited significant Mortality after 24 hrs and 48 hrs in both the mosquito species viz. *Aedes aegypti* and *Culex quiquefasiatus* at 500, 250 and 100 ppm doses.
- In case of *Aedes aegypti* at 500 and 250 ppm doses, **A_1** extract exhibited 100% kill within 24 hrs whereas at 100 ppm, kill was observed after 48 hrs.
- In case of **A_2** extract, 100% kill was achieved after 48 hrs at 500 ppm.
- In case of *Culex quiquefasiatus* at 500, 250 and 100 ppm doses, **A_1** extract gave 92-95 % mortality within 48 hrs whereas **A_2** exhibited 81- 84 % mortality.
- In case of *Aedes aegypti*, extracts **C_1** exhibited 91.33% mortality at 500 ppm after 48 hrs.
- Other extracts against both mosquito larvae, the mortality was less than 100%.

Thus from larvicidal activity of out of four plant species, *Himalayan Doop* (A) and *Sterculia foetida* (C) showed significant activity.

Column Chromatography Exteriments

Himalayan Doop (A) and *Sterculia foetida* (C) showed significant larvicidal activity among the four medicinal plants. Isolation of active ingradients from active extracts by column chromatography was carried out.

The extract **A1 (10.59gm)** was fractioned over silica gel (115g) column (60×1.5) starting with Hexane and continuing with successive increase percentage of ethyl acetate followed by mixture of ethyl acetate-methanol and finally with Methanol. The fractions were collected according to the colored bands and 250ml of volume. The progress of column chromatographic separation was monitored by performing thin layer chromatography of

each fraction. Fraction showing similar composition by TLC were combined together to obtain (13) major fractions.

Compound-**1** (0.80 g) obtained from Column fraction no.1, eluted from hexane (100 %), yielded yellow fatty mass shows single spot to TLC and GCMS. In GCMS C-I shows M^+ peak at 280.8 and Base peak at 161.IR spectrum of compound **1**, shows broad peak at 3370.29 cm^{-1} indicates presence of –OH functional group., 2953.83-2852.96 cm^{-1} shows strong Alkane C-H streching, ^{1}H-NMR spectrum of compound **1**, from chemical shift which shows long chain compound.

The methanol Extract 'C$_4$' (l2.13g) was fractioned over silica gel cc (3.5x 70) starting with Hexane and continuing in with successive increase percentage of ethyl acetate followed by mixture of ethyl acetate methanol and finally with methanol. The progress of column chromatographic separation was monitored by performing with thin later chromatography of fractions. Fractions showing similar composition were combined together to obtain ten major fractions.

Fraction 9 of column chromatography of extract 'C_4' shows single spot to TLC. Fraction 9 obtained as solid brown amorphous powder as compound II. Its melting is point above 240^{o}C. IR spectra shows characteristics peak at 3267.1(O-H stretching) cm $^{-1}$, 1607.20 and 1519.79 (aromatic C-H Stretching) $cm^{-1,}$ 1101.10 & 1048.63 (C-O stretching) cm^{-1}. The ^{1}H-NMR spectrum shows a characteristic peaks at 1.26 for presence of many CH_2 groups . Also at 4.10 it shows presence of ester (R-COOR). As its ^{1}H-NMR spectra is complicated may be presence of number of –OH group therefore its acylation has been carried out which gave compound III.

ACYLATION OF COMPOUND-II gave Compound III:

To the compound – II (0 .100 mg) acetic anhydride (excess, 0.5 ml) and drop of pyridine was added. The reaction mixture was allowed to stand overnight at room temperature under anhydrous conditions. Pyridine was removed under reduced pressure to yield compound – III (acyl derivative if compound-II)

Compound III, was obtained as brown amorphous solid. IR v_{max}-1740cm^{-1} indicate presence of acyl (-OCO-) group, 1227.82 & 1112.20 cm^{-1} indicate C-O stretching frequency.

- Compound-**I** obtained from Column fraction of potent extract of Himalyan doop plant. Compound-**1** eluted from hexane (100 %), yielded yellow fatty mass shows single spot to TLC and GCMS. In GCMS C-I shows M^+ peak at 280.8 and Base peak at 161.IR spectrum of compound **1**, shows broad peak at 3370.29 cm^{-1} indicates presence of –OH functional group. IR spectrum and ^{1}H-NMR spectra shows, compound –I, may be fatty long chain alcohol
- Compound-**II** obtained from Column fraction of potent extract of Sterculia foetida plant. Compound-II obtained as solid brown amorphous powder. Its melting is point above 240^o c. IR spectra shows characteristics peak at 3267.1 O-H stretching) cm^{-1}, 1607.20 and 1519.79 (aromatic C-H Stretching) $cm^{-1,}$ 1101.10 & 1048.63 (C-O stretching) cm^{-1}. The ^{1}H-NMR spectrum shows a characteristic peaks at 1.26 for presence of many CH_2 groups. Also at 4.10 it shows presence of ester (R-COOR).