EXPERIMENTS O1.1.2

SEPARATION METHODS I1

Pre-Lab
Briefly describe each of the following separating methods. Indicate the type or nature of mixtures which could best be separated by each method.

a) Column chromatography (C.C.)
b) Thin Layer chromatography (TLC)
c) Paper chromatography (PC)
d) Gas Liquid chromatography (GLC)
e) High Pressure (Performance) Liquid chromatography (HPLC)
f) Fractional distillation.
g) Distillation under reduced pressure
h) Steam distillation

Experiments
Extract the plant material provided with water and petroleum ether separately.
Perform paper chromatography and Thin layer Chromatography on each extract.
Determine the R_f values of the spots obtained

PAPER CHROMATOGRAPHY

THEORY:

Chromatography involves the separation of a mixture of substances flowing across or through a sorbing medium. The mixture is liquid or gaseous (fluid phase) and the sorbing medium (stationary phase) usually a liquid or liquid supported on a solid. Conditions are chosen such that components of the fluid phase interact reversibly with the stationary phase to different events, thus effecting a separation. Some factors affecting the degree of separation are solvent, stationary phase and temperature.

In paper chromatography, the mixture or unknown substance is spotted on a sheet of filter paper, and the solvent, often a mixture of aqueous organic solvents, is allowed to rise up the paper by capillary action. dissolving the unknown and rising to an arbitrary he. The paper is dried and the individual components identified by physical (e.g. UV light) or chemical (e.g. some chemical spray) methods For a given set of conditions (solvent, temperature, etc.) the relative rate of movement, r_f, is characteristic of a given compound. The R_f value is defined as

$$R_f = \frac{\text{distance the compound moves}}{\text{distance the solvent moves}}$$

since each compound has a characteristic R_f value, this method can also be used for determining the identity of an unknown compound. In this experiment you will measure the R_f values for a number of known amino acids and use these to determine the components of an unknown sample.