CHARACTERISATION OF METHANOLIC EXTRACT OF ROOTS OF *XYLIA XYLOCARPA*

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Abstract: *The medicinal plant* ***Xylia xylocarpa*** *syn:* ***Xylia dolobriformis*** *commonly known "Irul" belongs to the family Mimosaceae .The present work is based on the characterisation of the methanolic extract of roots was under taken. Physico-chemical analysis of the plant indicated the presence of alkaloidal components apart from anthocyanins. However, by the present investigation, alkaloidal components could not be isolated. Since very few components have been reported from the various parts of* ***Xylia xylocarpa,*** *and fractionation of the root extracts revealed a number of fluorescent spots on TLC and PC. Hence,the present study has proved the usefulness of Xylia xylocarpa for medicinal values and phytochemicals indicates its potential as a source of useful drugs.*

Keywords: *Xylia xylocarpa , characterisation, methanol, TLC, PC and fluorescent spots.*

1. INTRODUCTION:

Xylia xylocarpa **syn:** ***Xylia dolobriformis*** commonly known Irul belongs to the family **Mimosaceae.**It is a medicinal plant which is grown in tropical or subtropical regions. It normally grows to a height of 30 feet. The legume genus *Xylia* comprises of two species via *Xylia kerrii* and *Xylia longipes*. In general, the nomenclature confusion in the genus *Xylia* .On the basis of herbarium studies, it was concluded that the Indian species is ***Xylia xylocarpa*** and the Burmese species is *Xylia kerrii* and not *Xylia dolobriformis* [1]. Pharmacological investigation of *Xylia longipes* revealed the presence of a new antifungal compounds Xylamide and other secondary metabolites [2]. The present work is based on the characterisation of methanolic extract of roots of ***Xylia xylocarpa***.

2. LITERATURE REVIEW:

Decoction of the bark and leaves was used for treating leprosy, vomiting, diarrhoea, gonorrhoea, and ulcers. Generally, the oil from seeds is used in treating rhematism, piles and leprosy. The wood of *Xylia xylocarpa* was used for construction work, for railway sleepers, in ship buildings and in bridges [3].Phytochemical investigations on various parts of *Xylia xylocarpa* have been reported in the past. The isolation of heartwood constituents namely manoyl oxide,3-oxo manoyl oxide, sandaracopimaradiene, sandaracopimaradien- 3-one, sandaracopimaradien-3β,18-diol from *Xylia dolobriformis* [4].Acetone extract of stem bark yielded an amorphous light pinkish homogeneous compound,designated as dolopriproanthocyanidin[5]. The isolation of leaves constituent namely Trans hydroxyl pipecolic acid [6].

3. MATERIALS AND METHODS:

3.1 Collection of the plant material

The root material of ***Xylia xylocarpa*** was collected from the nearby Kollihills.

3.2 Pretreatment of the plant material

The material was air dried under controlled conditions to avoid too many chemical changes occurring.

Extraction

The extraction process involved under the following operations namely i) Comminution ii) Maceration and Percolation and iii) Distillation.

3.3 Preparation of the methanolic extract of roots

The air-dried pieces of roots (500g) of ***Xylia xylocarpa*** were extracted with methanol (3x2L) for a period of 3x6hrs. The blood red coloured extract obtained by filtration and concentrated by distillation. By this, the bulky solution was concentrated into a small volume. As a standard precaution against the loss of material, the concentrated extract was stored in a refrigerator.

3.4 Investigation of the methanolic extract of roots

The concentrated extract tends to deposit a dark brown solid on standing which was collected by filtration. The filtrate that gives fruity smell was subjected to the following preliminary tests [7].

i) Ferric test: To the filtrate, 2-3 drops of ferric chloride was added and colour was noted.

ii) Salkowski test: To the filtrate, 1ml of chloroform was added and few drops of concentrated sulphuric acid was added slowly along the sides of the test tube.
iii) Dragendorff test: To the filtrate, 2 drops of Dragendorff reagent was added and colour was noted.

3.5 TLC analysis of the methanolic extract

The extract was also subjected to TLC analysis in various solvent systems.The results were noted. A layer of flaky white substance was observed floating in the extract. It was separated, dissolved in alcohol and the solution was stored in the refrigerator. A light brown solid that separated was collected by filtration. TLC tested it for homogeneity with benzene as solvent system. A dark brown solid that was collected by filtration of the concentrated methanolic extract was analysed. It was designated as XX1.

3.6 Analysis of the compound XX1 from methanolic extract

The compound XX1 was analysed as given below:
i) Flame test: The substance was heated in a non-luminous flame.
ii) TLC analysis : Adsordent - Silica gel; Solvent system- Chloroform

3.7 Acetylation and Methylation reaction for the compound XX1

Acetylation reaction:

The solid XX1 (100mg) in acetic anhydride (5ml) and pyridine (1ml) was left for 48 hrs at room temperature. On dilution, a solid separated which crystallized from aqueous methanol. The light brown solid that separated was filtered and dried.

Methylation reaction:

The solid XX1 (500mg) was refluxed with dimethyl sulphate (1ml) ,acetone (125ml) and potassium carbonate (7.5g) for 24 hrs. The product obtained after removing acetone was treated with water when a small amount of solid separated out.The methyl ether was purified by column chromatography using silica gel, eluting successively with petroleum ether (60-80^0 C) and benzene. A pale yellow solid was obtained from the petroleum ether fraction and it was recrystallized from ethanol.

3.8 Fractionation of filtrate

The concentrated filtrate was macerated with water and the aqueous solution was fractionated [8]. The aqueous portion was successively extracted with various extracts in equal proportions (3x100ml).The extracts were subjected to TLC analysis is given in Table 1.

Table 1. TLC system used for the analysis of various extracts
Adsorbent used : Silica gel (G for TLC)

S.NO	Extract	Solvent system
1.	Petroleum ether (60-80^{0}C)	Benzene
2.	Benzene	Benzene
3.	Chloroform	Benzene : Ethyl acetate (4:1)
4.	Acetone	Ethyl acetate : Benzene (4:1)
5.	Ethyl acetate	Ethyl acetate : Benzene (4:1)
6.	Alcohol	Ethyl acetate : Benzene (4:1)
7.	Ether	Benzene : Ethyl acetate (4:1)
8.	Dichloromethane	Ethyl acetate : Benzene (4:1)

The extracts were evaporated separately, the residues were analysed, and results noted. The petroleum ether extract yielded a dull white solid designated as XX2, which was analysed.

3.9 Analysis of aqueous portion

After fractionation, the remaining aqueous portion was concentrated under reduced pressure. A sticky reddish brown residue was refluxed with 1:1 HCl for 2hrs [9]. The hydrolysed fraction was pink in colour. It was tested for the presence of anthocyanins.

4. RESULTS AND DISCUSSION:

The results of the characterisation of the chemical constituents of the methanolic extract of roots of ***Xylia xylocarpa*** are presented herewith and discussed in the light of objectives set forth.

4.1 Results of the preliminary tests of the filtrate of methanolic extract

- **Ferric test**: The filtrate did not give any characteristic colour with ferric chloride implying absence of free phenolics.
- **Salkowski test**: When Salkowski reagent was added to the filtrate, no colour was observed which infers the absence of terpenoidal compounds.
- **Dragendorff test:** On adding Dragendorff reagent to the filtrate, an orange colour was produced which indicates the presence of alkaloidal compounds.

4.2 TLC analysis of the methanolic extract

The following Table 2 shows the results of the TLC analysis of the extract in various solvent systems. TLC analysis of the flaky white substance indicated one yellowish green fluorescent spot. However, sufficient quantity of substance could not be isolated for further analysis.

Table 2. TLC of the methanolic extract in various solvent systems.

S.NO	Solvent system	Fluorescence in UV	Number of spots in iodine	R_f
1.	Benzene	-	-	-
2.	Benzene + Ethyl acetate (1:1)	-	1	0.13
3.	Ethyl acetate	-	2	0.33 and 0.64
4.	Chloroform	-	-	-
5.	Chloroform + methanol (9:1)	-	-	-
6.	Benzene +Alcohol(9:1)	-	-	-

4.3 Results of the analysis of the compound XX1 are given below:

- **Flame test:** The compound did not give a sooty flame. Therefore, it is aliphatic.
- **TLC analysis:** It revealed a number of components.The solid XX1 could not be characterized as it was unresolvable by TLC. All stages to characterize it by acetylation and methylation reactions also did not succeed since sufficient amount of homogeneous compounds were not obtained**.**

4.4 Fractionation of filtrate:

The results of the TLC analysis of the various fractions obtained by fractionation are tabulated in Table 3.

Table 3. Results of the TLC analysis of the various extracts

S.NO	Extract	Solvent system	Number of spots in iodine	Fluorescence in UV
1.	Petroleum ether (60-80^{0}C)	Benzene	2	Blue green
2.	Benzene	Benzene	2	Green
3.	Chloroform	Benzene : Ethyl acetate (4:1)	4	Blue and green
4.	Acetone	Ethyl acetate : Benzene (4:1)	3	Green and blue
5.	Ethyl acetate	Ethyl acetate : Benzene (4:1)	3	Yellowish green
6.	Alcohol	Ethyl acetate : Benzene (4:1)	3	Blue
7.	Ether	Benzene : Ethyl acetate (4:1)	3	Blue
8.	Dichloromethane	Ethyl acetate : Benzene (4:1)	2	Blue

The residues obtained from the fractionated portions were analysed. The Petroleum ether (60-80^{0}C) fraction gave dull white solid (XX2).Its melting point was 97-100^{0}c.Yield of XX2 was 300mg.It tested positive for carbohydrates as proved by the following tests.

- Flame test: Black residue on heating in a non-luminous flame.
- Molisch test: Green ring was observed
- Phenyl hydrazine test: Crystalline yellow ozazone was formed.
- P.C.analysis: In BAW system (4:1:5) the compound XX1 gave one prominent spot. On spraying the paper chromatogram with 0.2%$KMnO_4$ and 1%Na_2CO_3 reagent.R_f = 0.70. Other fractions did not yield sufficient amount of any compound.

4.5 Analysis of aqueous portion

The hydrolysed aqueous fractions was tested for the presence of anthocyanins. With magnesium and hydrochloric acid, the extract gave red colour. A violet colour was observed with aqueous sodium hydroxide. These tests prove the presence of anthocyanins component present in the extract[10].

5. CONCLUSION AND RECOMMENDATIONS:

Xylia xylocarpa **syn:** ***Xylia dolobriformis*** is reported to be a medicinally important plant.Hence the phyto chemical investigation of the methanolic extract of roots was under taken and this extract was not reported earlier.Physico-chemical analysis of the plant indicated the presence of alkaloidal components apart from anthocyanins. However, by the present investigation, alkaloidal components could not be isolated. Since very few components have been reported from the various parts of ***Xylia xylocarpa,*** and fractionation of the root extracts revealed a number of fluorescent spots on TLC and PC, more of active components could be isolated in further investigations.

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