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Phytochemical Evaluation of *Eucalyptus citriodora*

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Abstract: *Eucalyptus citriodora* of Myrtaceae family was collected from Manzary Baba, Malakand Agency, NWFP Pakistan . After identification and post harvest treatment, a preliminary separation of constituents by TLC and best solvent for extraction was attempted. Further resolutions of the component of the crude extracts by TLC and R_f values suggested that two major compounds were present in the sample. Also an economically feasible procedure for the isolation of rutin, a glycoside, from Eucalyptus Citriodora has been developed.

Key words: *Eucalyptus citriodora*, TLC, R_f, rutin

Introduction

The history of physiological action and drug is very old. The world is rich in medicinal plants, growing wild or cultivated, forming a huge natural and economic wealth which must be protected, increased and industrialized for the sack of humanity, development of economy, wealth of the nations and health of peoples. Developing countries have a rich flora of medicinal plants that are potential sources of new drugs and new biologically active substances (Kirthar and Basu, 1993). International efforts and co-operation is needed to exploit these vast sources and the biological evaluation of the medicinal plants, taking care not to disturb the ecological balance, conserve land, preserve the germ plasma available and their forest wealth.

Eucalyptus belongs to the family Myrtaceae, mostly found in tropical regions. Some plants of the family are of medicinal value (Pandey, 1987). In Pakistan, the Forest Department of Punjab in 1903, raised a small nursery of *E. globulus* (Siddiqui and Hussain, 1980). Rao and Redoly (1984), Del Morel (1967), Del Morel and Muller (1970) showed its inhibitory to annual herbs and toxicity to seed germination. Ragharaiah and jayardnaiah (1987) reported that essential oil from certain species of *Eucalyptus* showed anti fungal activity at different concentration against *Beanveria bassiana*. *Eucalyptus citriodora* has been known for being insect repellent effect (Menendez, 1992; Collen, 1993).

Yasinashita (1989) isolated rhaponticin, an antifouling substance from the leaves of *E. rubida*. Chopped leaves of *E. citriodora* produce reduction in nematode population (Akhtar and Alam, 1989). Keeping in view the importance of family and specially *E. citriodora*, an attempt has been made to develop a procedure / method for extraction of rutin, a phenolic compound (glycoside) resistant to boiling and fermentation and ingested daily more than one gram by human.

Material and Methods

Area Description: The plant was collected from Manzarray Baba, Malakand Agency, located at latitude 34-29^0 and longitude 71-45^0, at a distance of 120 km North of Peshawar city, the capital of NWFP, Pakistan and identified by specialized taxonomists of Botany Department and Islamia College, Peshawar University, Peshawar. Annual maximum temperature is 40^0 C in June - July and a minimum 0^0 C in December – January. Up to the 90% of the precipitation falls during monsoon and spring. The soil texture ranges from clay to course materials with a predominance of sands. *Eucalyptus citriodora* is a tall tree with erect stem, smooth bark, leaves 15-20 cm long, lanceolate, operculum hemispheric, stamens opening by slits and fruits 1.3 cm long. In Queen Land it is commonly known as the "lemon scented gum" or "spoken gum". It is cultivated as an ornamental tree, however in Java, South Africa, China, USSR and Congo, it is plant for commercial use to extract oil for manufacture of Menthol. *E. citriodora* oil was also found active against fungal strain (Qamar and Chaudhry, 1990 and Singh, 1992).

E. citriodora leaves, bark and seeds were dried. The dried specimen was ground to convert it in powder form and then kept in airtight bottles in order to avoid the adverse climatic effects and fungal attack. The powdered specimen was used during the experiments for the determination of active compounds. The extraction of any drug material with a solvent will yield a solution of the compounds, the composition of which will depend on the drug and the solvent used. The extract may contain a wide variety of compounds that plant materials may yield including, alkaloids, glycosides, phenolic proteins, oils, fats, and carbohydrates. To each 50 grams of powdered plant specimen, 100 ml of petroleum ether (C_2H_5-O-C_2H_5), chloroform ($CHCl_3$) and methanol (CH_3OH) were added in three different containers. All the three were shaken well and kept for 24 hours. The three extracts which were obtained were ultimately mixed, and concentrated by using rotary evaporator for chromatographic analysis. To 50 grams powdered plant, 100 ml (C_2H_5-O-C_2H_5) was added, shaken well, placed for 24 hours and extraction was made. The same procedure was followed for second extract with chloroform and third time with (CH_3OH. These three extracts were mixed, concentrated with rotary evaporator and used for chromatographic analysis.

Results and Discussions

TLC is used for separation of mixtures and identification of constituents using many solvents. Higher the retention speed or low the retention time on TLC plates, better the solvent would be and vice versa. A number of solvents and solvent mixtures (Table 1) were tried and found that mixture of ethyl acetate : petroleum ether: benzene (CH_3-CO-O-C_2H_5 : C_2H_5-O- C_2H_5 : C_6H_6) in ratio of 94 : 4 : 2 was found to be the best solvent for separation and from the elution system, seven fractions were isolated (Table 2). Table 2 shows that fraction 1, 2, and 3 are light brown in color while fractions 4 –7 are red in color. Moreover Table 3 confirmed the results of Table 2 in a sense that fraction 1 – 3 have almost the same R_f value i.e., 0.91 in C_2H_5-O-C_2H_5 : CH_3– CO-CH_3 1:1 ratio. The same fractions were tested in another system i.e., C_2H_5-O-C_2H_5 : CH_3OH in 3:1 with R_f value equal to 0.95, similarly color are also same. The result revealed that two type of compounds are confirmed in *Eucalyptus citriodora*.

Extraction of Rutin: Methanol : water (CH_3OH : H_2O) 3: 2 solvent system was used for extracting rutin from the specimen material. After extraction, filtration, concentration, the residue was dissolved in hot water because rutin, which is a glycoside, is soluble in water. After this benzene and chloroform were added for the removal of irrelevant materials like chlorophyll, oils, resins etc.

The isolation of glycosides in pure state from crude extracts is often carried out with multistage extraction and purification procedures. Normally neutral solvents are often used because they tend to hydrolyze in acidic condition. After extraction from plants, resolution of the

Table 1: Composition and resolution of developing system

Solvent System	Separation
Petroleum ether	Optimum
Chloroform	Satisfactory
Methanol	Little
n-Hexane	Little
Ethyl acetate	Significant
Petroleum ether : chloroform (1:1)	Satisfactory
Petroleum ether : chloroform (3:1)	Satisfactory
Ethyl acetate : petroleum ether (1:1)	Satisfactory
Acetone : petroleum ether (1:1)	Marginal solvent for the system
Ethyl acetate : petroleum ether : benzene (47:2:1)	Excellent
Ethyl acetate : petroleum ether : Benzene (47:1:2)	Good
Ethyl acetate : benzene (49:2)	Good

Table 2: Fractionation of sample in various eluting solvents

Solvent System	Color
Petroleum Ether : Chloroform (90 : 10)	Light Brown
Petroleum Ether : Chloroform (75 : 25)	Light Brown
Petroleum Ether : Chloroform (70 : 30)	Light Brown
Petroleum Ether : Chloroform (50 : 50)	Red
Petroleum Ether : Chloroform (40 : 60)	Red
Petroleum Ether : Chloroform (30 : 70)	Red
Petroleum Ether : Chloroform (20 : 80)	Red

Table 3: R_f values of the compounds isolated through the column chromatography

Color	Acetone: Petroleum Ether	Ethyl acetate : Methanol
	1:1	3:1
Light Brown	0.91	0.94
Light Brown	0.90	0.95
Light Brown	0.92	0.95
Red	0.88	0.92
Red	0.89	0.92
Red	0.89	0.93
Red	0.89	0.93

Table 4: R_f value of crude, sample and standard rutin

Sample	R_f Value	
	TLC $CH_3OH : CHCl_3$	Paper Chromatography $CH_3COOH : H_2O$
Crude	0.67	0.39
Sample	0.72	0.37
Standard	0.71	0.37

complex mixture into pure compounds requires the application of a number of other techniques like comparative TLC, preparative TLC, paper and column chromatography. Using $CHCl_3$, three fractions 1 –3 were obtained, combined, comparative TLC was conducted with rutin standard however presence of rutin was not established. By changing solvent ratio, fractions 4-8 and 9-13 were combined evaluated by comparative TLC but presence of rutin was not confirmed. However, fractions 14-20 collected with pure methanol CH_3OH on comparative TLC, with the standard rutin showed the presence of rutin. During the extraction of rutin, mild, conditions were chosen which prevented the decomposition of the glycoside. The TLC of isolated compound was carried out and R_f values were calculated.

From the R_f values (Table 4) of the sample it was confirmed that the material in question was rutin because the values were very close to that of the rutin standard. Similarly, the color of the scrapped material is also yellow similar to rutin standard. The melting point 188- 190^{0}C of rutin was also similar to the rutin standard.

Among the different solvent systems, the mixture of ethyl acetate-petroleum ether-benzene (94:4:2) appeared as an ideal solvent for the resolution of maximum number of constituents in a single step. Resolutions of crude extract by column chromatography yielded several fractions, among these fractions 1, 2, 3 exhibited the same color i.e. light brown and same R_f value. Similarly, fractions 4, 5, 6 and 7 also appeared red and possessed more or less the same R_f values. These observations suggested that only two major compounds have been extracted. For the extraction of rutin, from the dried plant material, methanol water (3:2) appeared as the best solvent that has selectively isolated rutin from the undesired constituent. Physio-chemical investigation (color, M.P., R_f etc., showed closer resemblance on the isolated product and standard rutin.

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