S. L. J. Tea Sci. 59 (1), 24-28, 1990. *Printed in Sri Lanka.*

AN IMPROVED PROCEDURE FOR THE ISOLATION OF FLAVANOLS FROM TEA LEAVES

P. A. N. Punyasiri, N. L. Herath and A. M. T. Amarakoon

(*Tea Research Institute of Sri Lanka, Talawakele, Sri Lanka*)

Although some procedures were developed in the past for the separation of flavanols in the fresh tea flush, they were time consuming, tedious and often the yield was not pure. In this study, an attempt has been made to isolate and purify tea flavanols using a relatively simple and an efficient procedure. Selective isolation of flavanols was achieved using a precipitation technique employing 0.1M lead acetate as the precipitating agent. Once isolated the six flavanols could be separated using 2-D cellulose thin layer chromatography, employing Butanol : Acetic Acid : Water (6:1:2) and 2% acetic acid as the solvent systems.

INTRODUCTION

Tea is a perennial crop grown mainly in tropical and sub-tropical countries and is of economic importance to Sri Lanka. Sanderson (1972) has made a detailed study of this plant with special reference to the chemistry and biochemistry of tea.

Chemical analysis has shown that amongst the numerous groups of chemical compounds found in the fresh tea flush, polyphenols are the most predominant group with amounts ranging from 25-35% on a dry weight basis (Sanderson, 1972). These polyphenols include flavanols (cetechins), flavonols and their glycosides, leucoanthocyanins and phenolic acids. Of these, flavanols comprise the highest percentage (Millin, Crispin and Swain, 1969) (Table 1).

TABLE 1 - *Amount of flavanols in tea flush on a % dry weight basis*

Flavanols	*Amount in flush*
(–) - Catechin	1 - 2
(+) - Gallocatechin	3 - 4
(–) - Epicatechin	1 - 3
(–) - Epigallocatechin gallate	9 - 13
(–) - Epicatechin gallate	3 - 6
(–) - Epigallocatechin	3 - 6

During the manufacture of black tea, these flavanols in particular undergo oxidative changes to produce a group of compounds (theaflavins and thearubigins) that contribute to colour, quality and character in black tea.

Several attempts were made in the past for the separation of tea leaf flavanols, which included paper chromatography (Roberts and Wood, 1951) and thin layer chromatography (Forrest and Bendall, 1969). However due to the long period of time involved in the former while contaminating substances overlap with flavanols in the latter, these procedures have not received wide recognition. Further, owing to the highly reactive nature of these polyphenols, development of improved methods of separation has been relatively slow. This paper reports a procedure by which flavanols found in fresh tea flush are separated by the combined methods of selective precipitation and thin layer chromatography.

MATERIALS AND METHODS

Extraction of polyphenols

Fresh tea flush (25 g, clone DT1) was plunged into boiling distilled water (150 ml) and allowed to boil for 10 min. This mixture was allowed to cool to room temperature and filtered through Whatman No. 42 filter paper.

Lead acetate precipitation

The filtrate was treated dropwise with 0.1M lead acetate until the formation of the precipitate was complete. This solution (along with the precipitate) was next centrifuged (4000 rpm) for 10 min and the supernatant decanted and tested for the presence of any polyphenols.

The precipitate was washed 3 times with distilled water to remove any free lead acetate or impurities, centrifuged (4000 rpm) and treated with 0.1M HCl (250 ml), when it totally went into solution. This solution was next extracted 6 times with ethyl acetate (100 ml each) and the pooled fraction was dried in a rotary evaporator (55°C).

Cellulose thin layer chromatography (TLC)

A known quantity of the above residue (5 mg) was dissolved in 2 ml methanol and a small aliquot (20 µl) was spotted on a cellulose TLC plate (Cellulose DS-0 Fluka Chemie AG; 20 x 20 cm). Similarly a known quantity of the crude extract was spotted on cellulose TLC plate. A two dimensional development of the plates was carried out using Butanol : Acetic Acid: Water (6:1:2) in the first direction and 2% Acetic Acid in the second direction. After the development, the plates were sprayed with diazotized p-nitroaniline reagent to locate flavanols. The spots were identified by running authentic markers on separate plates and comparing their R_f values.

RESULTS AND DISCUSSION

The hot water extract of fresh tea flush would have extracted polyphenols including flavanols, flavonols and their glycosides, phenolic acids and leucoanthocyanins into the medium. Along with these, amino acids, carbohydrates and purines would also have been extracted as they too are soluble in boiling water. Their presence was confirmed by observing the appearance of reaction products when treated with reagents specific for these groups of compounds in the above extract. Re-extraction of leaves only served to extract large amounts of impurities along with negligible amounts of polyphenols.

Lead acetate has been used in the past for the precipitation of polyphenols from plant materials (Seikel, 1964). However this is the first instance where polyphenols from fresh tea flush was precipitated using the above method. The precipitate was confirmed to be formed of lead acetate-polyphenol complex as was apparent from the absence of any detectable polyphenols in the supernatant. It is assumed that the above precipitate was formed through co-ordination interactions between polyphenols and lead acetate (Fig. 1). However, it should be noted that the type of polyphenols which interact with lead acetate are only those that contain hydroxyl groups in the ortho position (orthodihydroxy polyphenols). Therefore polyphenols such as phenolic acids found in trace amounts in the fresh tea flush were not precipitated while all the six flavanols which are instrumental in contributing to colour, quality and character in made tea were precipitated with lead acetate as all contained hydroxyl groups in the ortho position. Along with these, flavanols found in the fresh tea flush such as quercetin myrecetin and leucoanthocyanins were also precipitated for the same reason.

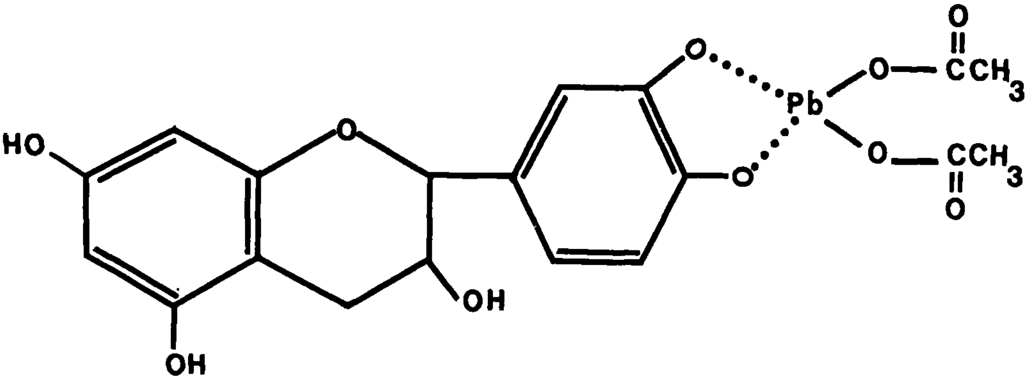

Fig.1 – *Lead acetate - (+) - Catechin Complex*

When the solution obtained by treating with 0.1M HCl was assessed for polyphenols (Swain and Hills, 1959) the quantities detected were representative of what was precipitated with lead acetate, thereby confirming that all bound polyphenols have been liberated from the above complex. This solution however was free of any carbohydrates, amino acids and purines when tested using specific reagents. The above method therefore has selectively precipitated the polyphenols (mainly flavanols) leaving behind the impurities in the supernatant.

The extraction of the above solution with ethyl acetate resulted in the selective removal of flavanols into the solvent layer leaving the lead acetate in the aqueous extract. The yellowish white powder that was obtained when the solvent layer was taken into dryness was confirmed to be polyphenols when tested with 1% vanillin reagent.

Subsequent to the two dimensional development the six distinct spots were identified as the flavanols of interest (Fig. 2a) on the basis of their R_f values and spectral data. The effectiveness in the lead acetate purification procedure was noticed by comparing the results with that of the TLC where an aliquot of the crude extract was subjected to separation (Fig. 2b).

The above procedure therefore could be adapted as an effective method in the separation of tea flavanols. The total separation could be achieved in a period of 8 h. This procedure is specially useful to laboratories which are not instrument oriented and have to carry out research with limited facilities.

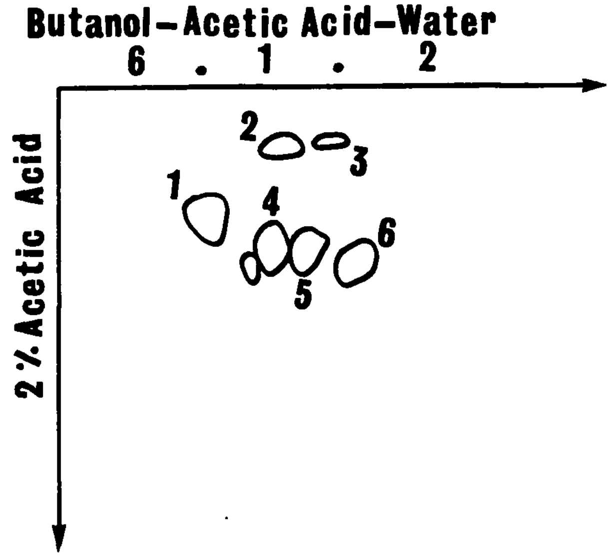

Fig. 2a – *TLC separation of purified extract*

1. (-) - Epigallocatechin
2. (-) - Epigallocatechin gallate
3. (-) - Epicatechin gallate
4. (+) - Gallocatechin
5. (-) - Epicatechin
6. (+) - Catechin

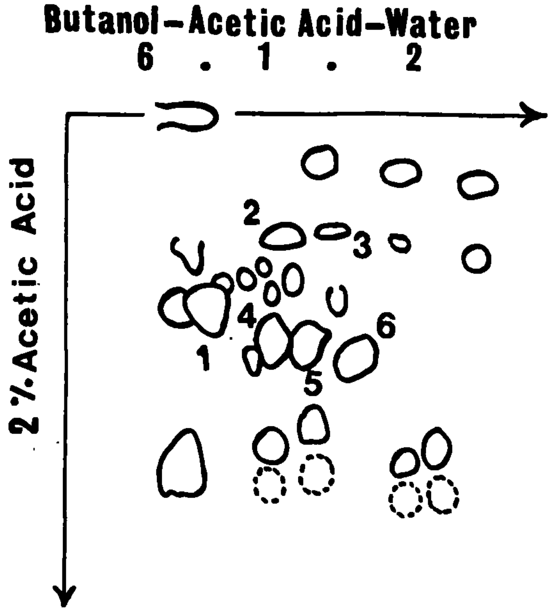

Fig. 2 b – *TLC separation of crude extract* (*Identification of numbered spots as in Fig. 2a*)

REFERENCES

FORREST, G. I. and BENDALL, E. (1960). The distribution of polyphenols in the tea plant (*Camellia sinensis L.*) *Biochem J.* 113. 741 - 745.

MILLIN, D. J., CRISPIN, D. J. and SWAIN, D. (1969). Non-volatile compounds of black tea and their contribution to the character of beverage. *J. Sci. Fd. Agric.* 17, 717 - 722.

ROBERTS, E. A. H. and WOOD, D. J. (1951). A study of polyphenols in tea leaf by paper chromatography. *Biochem, J.* 49, 414 - 422.

SANDERSON, G. W. (1972). The chemistry of tea and tea manufacturing. In "Structural and Functional Aspects of Phytochemistry". (ed. V. C. Runeckles and T. C. Tso) . 5, 247 - 306. Academic Press, New York.

SEIKEL, M. K. (1964). Isolation and identification of phenolic compounds in biological material. In "Biochemistry of Phenolic Compounds" (ed . J.B. Harbone). 34-76, Academic Press, London, pp 618.

SWAIN, T. and HILLS, W. E. (1959). The phenolic constituents of *Prunus domestica.* 1. The quantitative analysis of phenolic constituents. *J. Sci. Fd. Agric.* 10, 423 - 439.