

ALKALOIDS ANALYSIS IN ROOT AND LEAF FRACTIONS OF SARPAGANDHA (*RAUWOLFIA SERPENTINA*)

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ABSTRACT

Rauwolfia is cultivated for the medicinal use which is due to its active ingredients (50 alkaloids particularly reserpine). Reserpine and serpentine were confirmed and identified by chemical test and Thin Layer Chromatography. Crude alkaloid content was found to be 0.416 mg/gm and 0.217 mg/g on dry weight basis in root and leaves of rauwolfia plant. Among these alkaloids reserpine was found abundantly. Amount of alkaloids were higher in root fraction as compared to leaf.

Keywords: Sarpagandha, *Rauwolfia serpentina*, Reserpine, Serpentine, Alkaloids

The plant is a biosynthetic laboratory, not only for chemical compounds, but also a multitude of compounds like glycosides, alkaloids etc. These exert physiological and therapeutic effects. Medicinal herbs constitute an effective source of traditional (Ayurvedic, Unani and Homeopathy) and modern medicine. Plants have been an important source of medicine for thousands of years. There are a number of synthetic medicines which have been derived from medicinal herbs; Digoxin, Aspirin, Ephedrine, Quinine, Vincristine, Vinblastine, Taxoi Artemisinin, Hypericin, Silymarin are some examples.

Rauwolfia serpentina (family: Apocynaceae) is a medicinally important herb (Salma *et al.*, 2008). Roots and leaves are major plant parts containing medicinal properties due to presence of alkaloids. This plant is mainly used as a source of reserpine but now, more than 50 different alkaloids have been isolated from this plant (Klyushnichenko *et al.*, 1995), which are used to treat hypertension (Von Poser *et al.*, 1990) and breast cancer (Stanford *et al.*, 1986). Roots of this plant are also used for the treatment of many diseases such as insomnia, anxiety, excitement, schizophrenia, insanity, epilepsy, hypochondria, diarrhoea, dysentery etc in Ayurveda

medicines (Dastur, 1988; Kirtikar and Basu, 1993; Bhatara *et al.*, 1997; Ghani, 1998; Tona *et al.*, 1999). The root powder contains 0.15 to 0.2% of total alkaloids (mainly reserpine and serpentine) by weight. Reserpine is an alkaloid first isolated from *R. serpentina* which was widely used as an antihypertensive drug. Products of sarpagandha in Ayurveda are used in the form of sarpagandha ghanavati, sarpagandha yoga, sarpagandha chuma, mahesvari vati, kaphvatashmak, nidrajannan, raktbharshmak and javaghna. The herb is an effective drug in lowering blood pressure and to reduce fever. During delivery it is said to stimulate uterine contractions and promote the expulsion of tile fetus.

The chromatographic separation of secondary compounds and proteins for accessions characterization has been investigated (Jaswinder, 2001). Thin layer chromatography was used to analyze secondary compounds but now more advanced gas liquid Chromatography (GLC) or High performance liquid chromatography has been used for identification of compounds (Morgan, 1989). So, present study was focused to estimate crude alkaloid content by chemical methods and its fractionation by TLC.

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Extraction of alkaloids

Total alkaloids were estimated by extracting the alkaloids from root and leaves (Klyushnichenko *et al.*, 1995). Five grams of powdered dry samples were extracted thrice in methanol. The extracts were evaporated to dry under vacuum and it was dissolved in 100 ml of 0.01M HCL. The pH of filtered solutions was adjusted to 6.0 with 0.01M NaOH and used for further analysis. The crude extract obtained, was concentrated to yield crude alkaloid fraction. The research work was conducted in G.B. Pant Univ. of Agric. & Tech. Pantnagar, during 2009.

Alkaloid confirmation with chemical tests : Presence of alkaloids in plant material (root and leaf) was confirmed with the help of Mayer's and Wagner's reagents (Table 1).

1. Mayer's reagent: Solution A: The 1.36 g of $HgCl_2$ was dissolved in 60 mL of distilled water. Solution B: The 5 g of KI was dissolved in 10 mL water.

Both the solutions were mixed and make up the volume up to 100 mL.

2. Wagner's reagent: The 1.27 g Iodine and 2 g KI were dissolved in 5 mL of distilled water and make up the volume to 100 mL

Thin layer chromatography

TLC plates were prepared and activated at 110°C for 15 min. Methanolic extracts of sarpgandha roots and leaves were subjected at two places on silica coated plates. Chloroform: Methanol: Ammonium hydroxide (95:4.5:0.5) was taken as mobile phase. Spots

were developed by spraying with Dragondorff,s reagent. The same were intensify by spraying latter with 50% phosphoric acid.

Alkaloids from roots and leaves were extracted and the presence of alkaloids was confirmed by chemical tests (Table 1). Amount of crude alkaloids was 0.416 mg/g in roots, whereas it was 0.217 mg/g in leaf fraction on dry weight basis. In TLC analysis six spots/fractions were observed from root extract whereas, four from leaf extract. R_f values of major spots were calculated. Reserpine and serpentine were found in higher amount in both root and leaf fraction, having R_f values of 0.96 and 0.93 respectively. The relative percentage of active ingredients was lower in case of leaves as compared to roots (Fig 1 and 2).

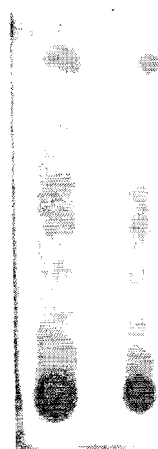


Fig 1 : TLC from root extract

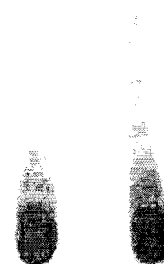


Fig 2 : TLC from leaf extract

Table 1: Chemical tests for alkaloids confirmation in crude extract of root and leaf

Experiment	Observation	Inference
1. Mayer's test: A few drops of this reagent were added in 1 mL of plant extract (root and leaf).	White precipitate forms	Confirmed the presence of alkaloids in the sample
2. Wagner's reagent: A few drops of this reagent were added in 1 mL of plant extract (root and leaf).	Brown flocculent precipitate forms	Confirmed the presence of alkaloids in the sample

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