

ABSTRACT

The present investigation has been carried out with *Fagopyrum dibotrys* (D. Don) Hara of the Darjeeling and Gangtok varieties, the ethnomedicine, which are growing in the wild condition. Attempts have been made to investigate the valid name of *Fagopyrum dibotrys* (D. Don) Hara. It is studied after comparing the authentic specimens with the herbarium sheets of *Fagopyrum dibotrys* (D. Don) Hara deposited in Botanical Survey of India, Central National Herbarium Section, and Howrah.

Isolation and characterization of the flavonoids, phytosterol and phenolic acids from two varieties of the plant growing in the region have been carried out for understanding their chemical make up. It helped to study the taxa on the chemical basis and also for commercial utilisation of their natural products.

Phytochemical analysis of *Fagopyrum dibotrys* (D. Don) Hara of the Darjeeling and Gangtok varieties has been performed following conventional method of soxhleting air-dried plant parts first with petroleum ether followed by benzene, chloroform methanol and their mixtures extract. Crude petroleum ether has been column chromatographed over alumina and pure crystals have been isolated after elution with suitable solvent. Methanolic extract has been separated into phenolic acid part, acid part, basic part and neutral part following conventional method and pure crystals have been separated from the sub-fraction from ether by column chromatography or fractional crystallization. Chemical constituents are identified after studying comparative behavior in m.p, m.m.p TLC, PC, spectrum of UV and IR of the isolated products with those of authentic samples.

Petroleum ether part of the leaves shows the presence of β sitosterol, lanosterol, stigmasterol and diosgenin. Phenolic acid part contained a considerable amount of residue identified as caffeic acid, benzoic acid, 3,4 dihydroxybenzoic acid, ferulic acid and o-coumaric acid. Basic part

showed no appreciable amount of solid to be identified. Acid part and neutral part show no appreciable amount of crystals.

Ether insoluble methanolic extract of leaf shows the presence of rutin, free quercetin, leucoanthocyanin, fagopyrin- like compound, cyanidin 3-glucoside and cyanidin 3,5 diglucoside. Water-soluble part showed the presence of saponin.

The presence of cyanidin 3 glucoside, free quercetin, fagopyrin like compound, lanosterol, stigmasterol, diosgenin, benzoic acid, 3,4 dihydroxybenzoic acid, o-coumaric acid and saponin may be considered as first time report from this plant. While working on the phytochemical analysis of different parts such as stem, rhizome and flower similar compounds have been isolated. Though in stem, the petroleum ether part shows the presence of stigmasterol, lanosterol and β sitosterol while in the ether insoluble fraction shows leucoanthocyanin, cyanidin 3-glucoside and cyanidin 3,5 diglucoside. The rhizome has been observed to contain phenolic acids as caffeic acid, benzoic acid, 3,4 dihydroxybenzoic acid, ferulic acid and o-coumaric acid and rutin in the ether insoluble part. The flower has been observed to contain fagopyrin like compound from ether insoluble part. No chemicals were isolated and worked out from the phenolic acid, acid, basic and neutral parts due to paucity of the chemicals. The methanolic part shows the presence of appreciable amount of crystals which were similar to rutin and leucoanthocyanin.

Besides their morphological difference, the varieties also differ in the presence of various phytochemicals from the qualitative as well as quantitative view. During the isolation and characterization of chemical constituents in various parts of *Fagopyrum dibotrys* (D.Don) Hara of the Darjeeling and Gangtok varieties, the former is characterized by the presence of anthocyanin, various phytosterol, phenolic acids and flavonoids. On the otherhand, no anthocyanin has been detected from the latter. The petroleum ether part of *Fagopyrum dibotrys* (D.Don) Hara of the Darjeeling and

Gangtok varieties has shown the presence of β sitosterol, lanosterol, stigmasterol and diosgenin. Phenolic acid part contains caffeic acid, o-coumaric acid, 3,4 dihydroxybenzoic acid, benzoic acid and ferulic acid. Rutin (Quercetin 3 rutinoside) and free quercetin have been detected in ether insoluble part. Other part of methanolic part has been noted with cyanidin 3 glucoside, cyanidin 3,5 diglucoside and leucoanthocyanin. In the water-soluble part, the saponin that has been isolated and noted to be converted into 16 DPA after being subjected to acid hydrolysis.

The chemical constituents have been identified by the comparative studies of m.p, TLC, PC, spectrum of UV and IR with those of authentic samples.

A new method has been established for quantitative determination of rutin isolated from the leaves. Colour has been developed in the presence of 2 % FeCl_3 and measured at 575 nm. Following this method, the accumulation of rutin in the various parts has been observed. The maximum rutin contents in *Fagopyrum dibotrys* (D.Don) Hara has been estimated to be 5.2% and 7.0% in Darjeeling and Gangtok varieties, respectively.

It has been observed that the different stages of development of the leaf of *Fagopyrum dibotrys* (D.Don) Hara of the Darjeeling and Gangtok varieties show the different amount of flavonoidal compound. In order to have better understanding of metabolism of the flavonoid, investigation has been carried out to study leaf physiology with special emphasis on qualitative estimation of various biochemical parameters at the different developing stages.

The rapid increase in content of soluble and insoluble carbohydrate, at the initial phase of development of leaf is characteristics for *Fagopyrum dibotrys* (D.Don) Hara of the Darjeeling and Gangtok Varieties. Both the contents decline at the latter phase of development of leaves. On the otherhand, accumulation of total nitrogen has been noted to increase upto 5th month of development of leaf after that it gradually declines till senescent stage of the leaf. Free aminoacid has also been noted to decrease gradually as the plant

attains maturity. The protein content gradually increases upto 5th month of development of plant. After that all the contents decline .

The chlorophyll content has been found to increase upto 5th month and decline later on.

The trend in rise and fall of carbohydrate as well as chlorophyll seems that they are interrelated. The decrease in free aminoacid content is associated with increase of protein and RNA upto 5th month. This is justified for high requirement of enzyme protein during the biosynthesis of high amount of phenolic compound. There after gradual decrease of phenol content may be due to decrease in the protein and RNA content.

The enzymatic activity in the leaves also reflects the normal behavior to attain senescence of leaf.

Peroxidase activity has been observed to become maximum at the initial stage of the development of leaf upto 2nd month after that it has been observed to decline.

After the biochemical estimation, it has been observed that there may be a correlation amongst and the phenolic content, protein, aminoacids, total phenol and chlorophyll. It is believed that all the higher plant polyphenols are formed from phenylalanine via shikimic acid pathway the mechanism of which has been well reviewed. It is obvious that during the production of high content of phenolic compounds lead to decrease the aminoacid and the protein content. The percentage of phenol is the same in *Fagopyrum dibotrys* (D.Don) Hara of both Darjeeling and Gangtok varieties. The chlorophyll content has been estimated to be greater in Darjeeling varieties than Gangtok varieties, which might be a reason for more amount of carbohydrate in Darjeeling varieties than Gangtok varieties. Besides the estimation of biochemical parameter in the leaves, the estimation of natural products has also been taken into consideration. The isolated natural products such as anthocyanin and leucoanthocyanin have been estimated in *Fagopyrum dibotrys* (D.Don) Hara of both Darjeeling and Gangtok varieties .The percentage of anthocyanin and leucoanthocyanin have been noted to be

2.9% and 3.9 % in Darjeeling variety, respectively. While the contents of anthocyanin is nil in the Gangtok variety but the 5% of leucoanthocyanin has observed to be present in the variety.

The effect of various micronutrients and NPK of the different combinations on the growth performances of the plant and other various growth parameters have also been observed in *Fagopyrum dibotrys* (D. Don) Hara of the Darjeeling and Gangtok varieties at NBU campus.

In connection with the study in the effect of various NPK combinations on *Fagopyrum dibotrys* (D. Don) Hara of both Darjeeling and Gangtok varieties – K culture solution, PK culture flasks observed to be the best for Gangtok variety but –N culture solution treated plant does not show good result as compared to other culture solutions. The different concentrations such as 10^{-3} to 10^{-7} M of $ZnCl_2$, $CuCl_2$ and $MnCl_2$ have been prepared for the growth of *Fagopyrum dibotrys* (D. Don) Hara of both Darjeeling and Gangtok varieties.

While working on their morphological variation, it has been noted that these two varieties are very similar in appearance and may be distinguished by certain characters such as colour of leaf, stem and flowers. These characters were studied through macroscopic and microscopic studies. Morphological studies of *Fagopyrum dibotrys* (D. Don) Hara of the Darjeeling and Gangtok varieties have been studied in Darjeeling, Gangtok and N.B.U campus.

Growth of *Fagopyrum dibotrys* (D. Don) Hara of the Darjeeling and Gangtok varieties have been observed to be stunted with less number of the leaves and no flower in the N.B.U campus condition. Several factors such as temperature and soil moisture etc are supposed to determine the normal growth of *Fagopyrum dibotrys* (D. Don) Hara. So, both varieties have been studied in different soil moisture condition at N.B.U campus.

In the macroscopic observation, the variation in the number of stamens has been observed. While in the microscopic study, the variation in the dump-bell shaped vascular bundle and I shaped vascular bundle in *Fagopyrum dibotrys* (D. Don) Hara of both Darjeeling and Gangtok varieties, respectively have

been observed. It is, therefore, may be treated to be the first time report from these plants.

During survey in the region of Darjeeling and Sikkim Himalayas, it has been observed that the local people commonly use *Fagopyrum dibotrys* (D.Don) Hara as an ethnomedicine. This ethnomedicinal practice has long been persisted in these areas. The old literature has also been surveyed and the possible trend of the development of traditional system of medical practice in Darjeeling and Sikkim studied. Besides an attempt has been made with special emphasis on scientific evaluation of the plant. So, the different concentrations of water soluble methanol extract of *Fagopyrum dibotrys* (D.Don) Hara were prepared and injected intravenously into albino rat. After four days of intravenous injection of 50 µg, 100µg, 200µg, 500µg and 1000 µg of water soluble methanol extract of each of the Darjeeling and Gangtok varieties of *Fagopyrum dibotrys* (D.Don) Hara separately more or less the same trend such as 39 %, 30 %, 58 %, 40 % and 80 % of the activation of T-lymphocytes have been observed. Though the water soluble methanol extract of 1000 µg has maximum activation percentage, but the concentration has been not suitable for immunological development of the animals. 200 µg of water extract found suitable for the immunological development of the animals. The bilirubin was also estimated from the blood of patients who were taking 5.8 gm dried leaves extract twice a day as advice of the tribal medicine men and on the 13th day of treatment, decreased in the bilirubin content has been observed. Antifungal activities were also observed with the rhizome water extract of *Fagopyrum dibotrys* (D.Don) Hara of the Darjeeling and Gangtok varieties. This show that this ethnomedicines have the potentially to fight against the diseases.

The information of ethnomedicinal practises of Darjeeling and Sikkim Himalayas has also been collected from the priest doctors and on the basis of which the baid practice in Sikkim has been worked out.

Besides, an attempt has been made to investigate the plant with a biotechnological approach. The use of modified Murashige and Skoog medium has been observed to give good result. A suitable composition of a medium for the suspension culture of stem explant of *Fagopyrum dibotrys* (D. Don) Hara has been worked out. The different natural products such as β sitosterol, leucoanthocyanin, cyanidin 3 glucoside; rutin and saponin have been isolated and identified after 60 days of initiation of suspension culture of the explants.