Qualitative and Quantitative estimation of major chemical constituents of some common species of *Cassia* seeds

Suman Kumari, Anuj Rani, Brajesh Kumar Singh, and B.K. Singh

University department of Botany, T.M. Bhagalpur University, Bhagalpur 812007, Bihar, India.

Abstract:

For the collection of seeds of *Cassia*, select different regions of Bihar representing eastern, western, southern, northern and central zones like Purnia, Muzaffarpur, Gaya, Patna, Lakhisarai, Munger and Bhagalpur directly from the place of occurrence. The seed samples was properly dried and stored separately for Qualitative and quantative estimation of amino acid, protein, sugar and alkaloids. During study eight amino acids present in seeds of *Cassia fistula* and five amino acids present in *Cassia occidentalis* seeds. Only seven amino acids were spotted in seeds of *Cassia sophera* whereas nine amino acids were present in seeds of *Cassia tora.* Qualitative estimation of protein by gel electrophoresis showed only four spots in the entire four species *Cassia fistula, Cassia occidentalis, Cassia sophera* and *Cassia tora*. Two different sugars were present in *Cassia fistula* and *Cassia occidentalis* seeds. In *Cassia sophera and Cassia tora* three sugar were present. Quantitative estimation of concentration of protein contents were maximum in *Cassia sophera* i.e., 49.04 μg/mg and minimum in *Cassia tora* i.e., 30.73 μg/mg. The concentration of protein in *Cassia occidentalis* and *Cassia fistula* were 43.37 and 48.12 μg/mg respectively. Quantitative estimation of total sugar in *Cassia fistula, Cassia occidentalis, Cassia sophera* and *Cassia tora* were done which were 173.46, 164.09, 164.85 and 199.54 μg/mg respectively. Maximum sugar concentration was found in *Cassia tora* and minimum in *Cassia occidentalis*. Maximum amount of alkaloid were found present in *Cassia fistula* i.e., 2.5% and minimum in *Cassia occidentalis* i.e., 1.5%. The amount of alkaloid present in *Cassia sophera* and *Cassia tora* were 1.51% and 2.0% respectively. Phenol was also quantitatively estimated, concentration of which was maximum in *Cassia fistula* seeds i.e., 0.173 μg/mg and minimum in *Cassia tora* i.e., 0.089 μg/mg. The phenolic concentration of *Cassia occidentalis* and *Cassia sophera* were 0.097 and 0.109 μg/mg respectively.

Key Word: Qualitative, quantitative, chemical constituents, *Cassia* seeds

Herbal drugs are crude preparations of various kinds of medicinal plants. In other words, herbal drugs is a dried medicinal plant or plant parts, such as leaf, stem, root, flower, fruit or seed

(Sukhdev, 1983). The genus "*Cassia*" is a member of the Fabaceae family (Leguminosae family) in the major group angiosperms (flowering plants). The Fabaceae or Leguminosae, commonly known as the legume, pea or bean family, is a large and economic important family of flowering plants. Plants of this family are found throughout the world, growing in many different environments and climates (Stevens, 200l).The plants range in habit from giant trees to small annual herbs, with the majority being herbaceous perennials. The plants have indeterminate inflorescences, which are sometimes reduced to a single flower. The flowers have a short hypanthium and a single carpel with a short gynophore, and after fertilization produce fruits that are legumes (Schrire *et al*., 2005). The leaves are usually alternate compound, and are even - or odd-pinnately compound. The name "Cassia" means "Cinnamon-like bark". In addition, the genus *Cassia* was for long ill-delimited with regards to the related Cassiinae - especially *Senna* (which has many medicinal important species).

The medicinal value of these plants lies on some chemical substances that produces a definite physiological effect of these substances are, alkaloids, flavonoids, glycosides, tannin oils, phenols and many others (Omaye, 2004). According to Kapur and Atal (1982), there is need that the local herbs be evaluated for phyto chemistry so as to determine the potential of indigenous sources of medicines. Many plants in this genus are used extensively in traditional medicine in tropical and warm subs tropical countries (Robber and Speedie, 1996). It is believed to possess a laxative effect. Its extract is reported to be beneficial in treating many skin diseases like eczema, rashes, ringworm etc. the seeds are roasted and boiled in water to produce tea as folk medicine (Perry, 1980). These plants have also been reported to treat constipation, common cold, fevers, intestinal disorders and in healing of wounds (Burkill, 1995).

MATERIAL AND METHOD:

Seeds of four medicinal plants viz., *Cassia fistula* Linn., (Amaltas), *Cassia occidentalis* Linn., (Chakban), *Cassia sophera* Linn., (Kasunda) and *Cassia tora* Linn., (Chakunda) were selected for the present investigations.

- Quantative estimation of protein, sugar and alkaloids Total phenol:
- Protein:

The soluble protein content was estimated by Folin Ciocalteau reagent (Lowry *et al*., 1951). 1 ml of seeds homogenate (0.1 gm / ml in Buffer solution) was taken in a centrifuge tube and 3 ml of 10% Trichloroacetic acid (TCA) was added. It was centrifuged for 20 minutes at 3000 rpm. The supernatant was discarded and centrifuge tube were left overnight in an inverted position. 5 ml 0.1 N NaOH was added to centrifuge tube and mixed with the help of cyclomixture. 0.5 ml of the above suspension was taken in a test tube and made the volume to 1 ml by distilled water (DW). Then 5 ml of alkaline copper sulphate reagent (50 parts of 2% Na_2CO_3 in 0.1 N NaOH and 1 part of 0.5% of $CuSO_4.5H_2O$ in 1% sodium potassium tartarate) was added to the above tube and

mixed thoroughly. Thereafter, 0.5 ml Folin Ciocalteau (F.C.) reagent was added and mixed. Finally, this mixture was allowed to stand for 30 minutes for optimum colour development and its absorbance was recorded at 600 nm against reagent blank.

The amount of protein was calculated against the standard curve of albumin and expressed as µg / mg seed on fresh weight basis. Data recorded at each stage are the mean of six different replicates for each sample. ANOVA test was made among various parameters at different stage of storage conditions and infested seed samples.

- Total sugar

Total sugar content was estimated according to the method of Dubois *et al*. (1956) using phenol sulphuric acid reagent. 2 ml seed sample (100 mg / 10 ml DW) was taken in a centrifuge tube; to which 2 ml each of 10% zinc sulphate and 2 ml of 0.5 N NaOH was added. The mixture was, then, centrifuged for 20 minutes at 3000 rpm. After centrifugation, 0.5 ml supernatant was taken in a test tube, to which 0.5 ml 5% aqueous phenol and 2.5 ml concentrated sulphuric acid was added slowly. The colour intensity was read at 490 nm against the reagent blank. The amount of total sugar was calculated with the help of standard curve of glucose and expressed as µg glucose / mg seed samples on fresh weight basis. Data recorded at each stage are the mean of six different replicates for each sample. ANOVA was made among various parameters at different stage of storage conditions and infested seed samples.

- Alkaloids

Alkaloid was estimated by the method of Mukherjee (1953). 100 gm of powdered seed sample was soaked with 80% Ammonium hydroxide solution and dried up. Subsequently, the seed sample was soxhlated with a mixture of chloroform and ethanol (3 : 1, v/v) for reflex on water bath for about 8 hrs. Then after it was filtered and added some extra amount of 3 : 1 v/v chloroform and ethanol. Stock solution was taken in separating funnel and added N/2 H_2SO_4 20 ml, 15 ml and 10 ml.

After that two layers were formed. Upper layer collected in the beaker was made alkaline with dilute NH_4OH until acidic solution was changed alkaline. The alkaloids were extracted from alkaline extract with 20 ml, 10 ml and 5 ml of pure chloroform. After that two layer was formed. Lower layer is taken and evaporated on water bath and dried up. The residue was weighed on monopan balance to calculate the crude alkaloids.

- Total phenol

Total phenol was estimated by following the method of Singh *et al.* (1978). 100 mg of powdered seed sample was homogenized with 10 ml of 80% ethanol in a glass homogenizer. The homogenate was centrifuged at 3000 rpm for 15 minutes. 1 ml of supernatant was diluted to 20 ml by adding glass distilled water. To this, 5 ml of freshly prepared colour reagent (a mixture of equal volume of 0.3% Ferric chloride in 0.4 N HCl and 0.3% Potassium Ferricyanide solution) was added. After 60 minutes, its optical density was recorded at 675 nm. The amount of total phenolic compound was calculated by comparing the readings with that of standard curve of tannin.

Qualitative estimation of amino acid, protein, sugar and alkaloids

- Free amino acid

For this purpose the ethanolic extract (10 mg seed sample per 1 ml in 80% ethanol) was subjected to thin layer chromatography (TLC). Free amino acids were estimated according to the method of Yemm and Cocking (1954). The ethanolic extract (10 mg / ml 80% ethanol) was subjected to TLC using n-butanol, acetic acid and glass distilled water (BAW 4 : 1 : 1 v/v/v) as solvent system. After running a distance of 15 cms, the TLC was air dried and sprayed with ninhydrin reagent (0.1 gm ninhydrin dissolved in 100 ml n-butanol and 1 ml glacial acetic acid). The sprayed TLC was incubated at 110°C for 5 minutes for colour development.

- Total sugar

Sugar contents were chromatographically separated following the method of Gauch *et al.* (1979). The ethanolic extract (10 mg / ml 80% ethanol) was subjected to thin layer chromatography (TLC) using butanol : acetic acid : water (4 : 1 : 1, v/v/v) as a solvent system. Thin layer chromatogram was run in the above system. After the run the dried chromatogram was sprayed with fresh Aniline phthalate solution. The dried TLC was incubated at 110°C for 10 minutes for colour development. Unknown sugar spots were characterized on the basis of comparison of colour and Rf value of standard sugar run simultaneously on the TLC.

- Total alkaloids

Alkaloid components were estimated according the method of Harborne (1973). The seed samples were grinded in powdered form and were used for extraction. 10% acetic acid in ethanol was added in the seed samples and left to stand for at least 4 hrs. The extract was concentrated one quarter of the original volume and precipitated the alkaloids by dropwise addition of conc. NH_4OH. Collected by centrifugation and washed with 1% NaOH. The residue was dissolved in a few drop of ethanol or chloroform. Loaded on TLC (silica gel G) plates and were run in solvent (methanol: conc. NH_4OH (200: 3). Presence of alkaloids on plate was detected first of all by any fluorescence in UV Light at wavelength of 254 nm.

- Protein

Protein estimation was done by gel electrophoresis. Salient features regarding preparation of gel, reservoir buffer and electrophoresis of seed samples were as below (Smith, 1976; Siciliano and Shaw, 1976).

The staining and destaining of the protein bands were done according to the method of Smith (1976). Prepared gels were up into the above staining solution for 10 – 20 minutes at room temperature and were subsequently changed to a destaining solution.

RESULTS AND DISCUSSION:

The nitrogen contents of seeds usually comprise, in addition to proteins, a certain amount of free amino acids and amides. Free amino acids, found in seeds, are usually the same as those forming parts of the protein structure. In addition, a few other amino acids and other heterocyclic amino acids have also been found in some seeds (Fowden, 1960 and Nee and Fowden, 1960). Qualitative estimation of free amino acids of seeds was determined by thin layer chromatographic technique. These were identified by comparing with standard samples.

Protein is one of the most important constituents of plant parts. Though a higher percentage of protein is found in reserve tissue of seeds, it may be present in the leaves roots, stems, floral parts, fruits, tubers etc. In seed, protein is present in the form of special bodies, termed as aleurone crystalloids etc.

Carbohydrates are polyhydroxy aldehydes or ketone derivatives. These containing aldehyde groups are called aldose sugars and ketone groups are called ketose sugars. These are classified as monosaccharides, oligosaccharide and polysaccharides.

Phenols, the secondary metabolites, which are necessary to sustain normal functioning of the life processes (Subba Rao *et al*., 1977), have long been implicated as active resistance factor in defense mechanism of plant against pathogen. Phenolic compounds embrace a considerable range of substances which possesses an aromatic ring bearing one or more hydroxyl subunits and are mostly of plant origin. Phenols and their products after oxidation are toxic to a variety of micro–organisms (Newton and Anderson, 1929).

Chopra *et al.* (1965); Wagner *et al.* (1984) and Oliver-Beever (1986) have given a comprehensive idea on the uses of plant alkaloids in the preparation of modern medicines. Besides this alkaloids have established disease resistance properties.

Qualitative estimation of amino acid, protein, sugar and alkaloids

Eight amino acids viz; lysine, threonine, valine, proline, glycine, tyrosine, trytophan and cysteine were found present in *Cassia fistula* seeds shown in Table – 01. In *Cassia occidentalis* seeds five amino acids spot i.e., lysine, threonine, cysteine, alanine and serine were spotted. Only seven amino acids were spotted in seeds of *Cassia sophera* i.e., lysine, threonine, proline, glycine, tyrosine, cysteine and alanine. Nine amino acids were present in seeds of *Cassia tora* i.e, lysine, threonine, glutamic acid, valine, proline, glycine, tryptophan, alanine and serine.

Qualitative estimation of protein by gel electrophoresis showed only four spots in the entire four species *Cassia fistula, Cassia occidentalis, Cassia sophera* and *Cassia tora*.

Table – 01 shows that two sugars were present in *Cassia fistula* seeds i.e., glucose and fructose. In *Cassia occidentalis* glucose and mannose sugars were recognised. In *Cassia sophera and Cassia tora* three spots were present i.e., of glucose, mannose and cellebiose. In qualitative estimation of alkaloid two spots were observed in *Cassia fistula, Cassia occidentalis, Cassia sophera* and *Cassia tora*.

Table 01: Qualitative estimation of Amino acid, Sugar, Protein, Alkaloid & Phenol

	Cassia fistula	*Cassia occidentalis*	*Cassia sophera*	*Cassia tora*
A.Amino acid				
Lysine	+	+	+	+
Threonine	+	+	+	+
Glutamic acid	-	-	-	+
Valine	+	-	-	+
Proline	+	-	+	+
Glycine	+	-	+	+
Tyrosine	+	-	+	-
Cysteine	+	+	+	-
Alanine	-	+	+	-
Tryptophan	+	-	-	+
Serine	-	+	-	+
B. Sugar				
Glucose	+	+	+	+
Mannose	-	+	+	+
Cellebiose	-	-	+	+
Fructose	+	-	-	-
C. Protein	+	+	+	+
D.Total alkaloids	+	+	+	+
E. Total phenol	+	+	+	+

Quantitative estimation

Table 02 showed quantative estimation of protein, sugar, alkaloid & phenol. Concentration of protein contents were maximum in *Cassia sophera* i.e., 49.04 μg/mg and minimum in *Cassia tora* i.e., 30.73 μg/mg. The concentration of protein in *Cassia occidentalis* and *Cassia fistula* were 43.37 and 48.12 μg/mg respectively.

Quantitative estimation of total sugar in *Cassia fistula, Cassia occidentalis, Cassia sophera* and *Cassia tora* were done which were 173.46, 164.09, 164.85 and 199.54 μg/mg respectively.

Maximum sugar concentration was found in *Cassia tora* and minimum in *Cassia occidentalis*.

Table 02: Quantative estimation of Protein, Sugar, Alkaloid & Phenol

Plant Name	Protein (μg/mg)	Total sugar (μg/mg)	Alkaloid (μg/mg)	Phenol (μg/mg)
Cassia fistula	48.12	173.46	2.50	0.173
Cassia occidentalis	43.37	164.09	1.50	0.097
Cassia sophera	49.04	164.85	1.51	0.109
Cassia tora	30.73	199.54	2.00	0.089

Maximum amount of alkaloid were found present in *Cassia fistula* i.e., 2.5% and minimum in *Cassia occidentalis* i.e., 1.5%. The amount of alkaloid present in *Cassia sophera* and *Cassia tora* were 1.51% and 2.0% respectively.

Phenol was also quantitatively estimated, concentration of which was maximum in *Cassia fistula* seeds i.e., 0.173 μg/mg and minimum in *Cassia tora* i.e., 0.089 μg/mg. The phenolic concentration of *Cassia occidentalis* and *Cassia sophera* were 0.097 and 0.109 μg/mg respectively.

Alkaloids having heterocyclic nitrogenous organic compounds (containing one or more nitrogen atoms) are widely distributed in plants and other natural resources. There are many synthetic substances which satisfy all the criteria of alkaloids except for the facts that they are not derived from biological resources.

Much attention has been paid by the biochemists to the biosynthesis of alkaloids. The most common precursors of alkaloids are amino acids. Picket and Court (1907) suggested that amino acids derived from protein breakdown, as the main precursor of alkaloids. This view was

supported by Sukhorukov and Borodulina (1932) and Weever (1933) while working with seedlings of *Datura* and *Ricinus* respectively. Besides, some proteins, amino acids, nicotinic acid and anthranilic acid may serve as nitrogen containing precursors for alkaloids biosynthesis (Groger, 1969; Luckner, 1969 and Fiedler *et al*., 1976). An overview on control mechanism in alkaloid biosynthesis is given by Floss *et al*, (1974).

Alkaloids are of interest mostly because of their physiological action and their uses in medicine and also, play a major role in plant resistance. Taubenhaus and Ezekiel (1936) first suggested that alkaloids are responsible for resistance in various plants.

The phenolic compounds are the natural plant products, which embraces a considerable range of substances and possesses an aromatic ring bearing one or more hydroxyl subunits. Importance of phenolic compounds in disease resistance has been recognized by many earlier investigators including Cook and Wilson (1915); Kuc (1957); Hare (1966); Biehn *et al*. (1968); Bhatia *et al*. (1972); Koradge *et al*. (1980) and Sharma *et al*. (1983) etc.

Besides disease resistance, phenolics also have been used for medicinal or pharmaceutical concerns. A large number of literatures are available regarding the role of phenolics as therapeutic compound (Goodman and Gillman, 1956; Williams, 1959; Jain, 1965; Weinges *et al*., 1971; Chakravarty *et al*., 1981; Mukherjee and Ghosh, 1972; Soulinna *et al*., 1975; Wagner, 1977).

Acknowledgment: I am thankful to Head University Department of Botany T.M. Bhagalpur University, Bhagalpur for providing laboratory facilities.

Reference:

1. Bhatia, J.S., D.S. Uppal and Bajaj (1972). Study of pheniolic contents of resistant and susceptible varieties of tomato (*Lycopersicum esculantum*) in relation to early blight diseases. *Indian Phytopath*. 55: 231 – 235.
2. Biehn, W.L., J. Kuc and E.B. Williams (1968). Accumulation of phenol in resistant plant fungi interactions. *Phytopathol*. 58: 1255 – 1260
3. Burkill, H. M. (1995). The Useful Plants of West Tropical Africa. Vol.3. Families J-L . Royal Botanic Garden, Kew. pp. 605.
4. Chakravarthy, B.K., S. Gupta, S.S. Gambhir and K.D. Gode (1981). 1 – Epicatechin – A normal antidiabatic drug. *Indian Drugs*. 18(5): 184 – 185.

5. Chopra, R.N., R.L. Badhwar and S. Ghosh (1965). Poisonous Plants of India. Vol I., I.C.A.R. Publication, New Delhi.
6. Chopra, R.N., R.L. Badhwar and S. Ghosh (1965). Poisonous Plants of India. Vol I., I.C.A.R. Publication, New Delhi.
7. Cook, M.J. and G.W. Wilson (1915). The influence of tannin contents of host plant on *Endothia parasitica* and related species. *Bot. Gaz*. 60: 346 – 361.
8. Fiedler, E., H.P. Fiedler, A. Gerhard, W. Keller-Schierlein, W.A. Konig, and H. Zahner (1976). Stoffwechsel produkte von micro-organism. 156 mitteilung. Synthese and biosynthese substituierler, Tryptanthrine. *Arch. Microbiol*. 107: 249.
9. Floss, H.G., E.J. Robers and P.F. Heinstein (1974). Regulatory control mechanisms in alkaloid biosynthesis. *Recent Adv. Phytochemic*. 8: 141 – 148.
10. Fowden, L. (1960). In, "The germination of seeds" by Mayer, A.M. and A.P. Mayber (1963). Pargamon Press : Oxford London, New York Paris. p. 27.
11. Goodman, L.S. and Gillman (1956). Pharmacological actions of phenolics. In : the Pharmacological basis of Therapeutics. MacMillan, New York.
12. Groger, D. (1969). Anthranilic acid as precursor of alkaloids. *Lloydia*. 32: 221 – 246.
13. Hare, R.C. (1966). Physiology of resistance to fungal diseases in plants. *Biot. Rev*. 32: 95 – 135.
14. Jain, S.K. (1965). International Monographs on Advanced Chemistry. 190 – 210.
15. Kapur, B. M. and Atal, C. K. (1982). Cultivation and Utilization of Medical Plants. Regional Research Laboratory Council of Scientific & Industrial Research, Jammu-Tawi, India. p. 877.
16. Koradge, B.K., P.D. Chavan and A.N. Thite (1980). Changes in phenolic compounds of tea leaves induced by powdery mildew infection. *Indian Phytopath*. 33(1): 114 – 116.
17. Kuc, J. (1957). A Biochemical study of the resistance of potato tuber tissue to attack by various fungi. *Phytopth. Z*. 47: 676 – 680.
18. Luckner, M. (1969). In "Biosythesis der Alkaloide Monthes, K. and Schutte, H.R. (eds.), Berlin, VEB Verlay der Wissenschafer. 510 – 550.
19. Mukherjee, K. and N.C. Ghosh (1972). *Coccinia indica* as potential hypoglycaemic agent. *Ind. J. Exp. Biol.* 10: 344 – 349.
20. Nayber (1963). Pergamom Press. Oxford London, New York, Paris. p. 27.
21. Nee, F.F. and L. Fowden (1960). In "The Germination of seeds" by Nayer, A.M. and A.P.
22. Newton, R. and J.A. Anderson (1929). Studies on the nature of rust resistance in wheat IV. Phenolic compounds of the wheat plants. *Canad. J. Res*. 1: 86 – 90.
23. Oliver – Bever, B. (1986). Medicinal plant in tropical west Africa. Cambridge University Press, Cambridge, London, New York, New Rochelle, Melbourne, Sydney.
24. Omaye, S. T. (2004). Food and Nutritional Toxicology. C. R. S. Press, USA. pp. 184-215.
25. Perry, M. L. (1980) Medicinal Plants of East and Southeast Asia: Attributes, Properties and Uses. MIT Press, Massachusetts, USA. p. 128.
26. Picket, A. and G. Court (1907). Sur quelques nouveaux alkaloids vegetaux. *Bull. Soc. Chem. France*. 4(1): 1001.
27. Robber, J. M. and Speedie, T. V. (1996). Pharmacognosy, Pharmacobiotechnology. Williams and Wilkins, Baltimore. pp 1-14.

28. Schrire, B. A., Lavin, M. and Lewis, G. P. (2005). "Global distribution patterns of the Leguminosae: Insights from recent phylogenies" In: I. Friis and H. Basley (Eds), Plant diversity and complexity patterns: Local, regional and global dimension. Biol. Skr., 55:375-38
29. Sharma, S.G., R. Narayan, S. Lal and C. Chaturvedi (1983). Role of phenolic compounds in resistance of maize of leaf blight caused by *Drechslera* state of *Cochliobolus heterostrophus*. *Indian Phytopath*. 36(1): 43 – 46.
30. Soulinna, E.M., R.N. Buchsbaum and E Racker (1975). *Canc. Res*. 35: 1865.
31. Stevens, P. F. (2001). Angiosperm Phylogeny. Website Version. www. mobot. org.
32. SubbaRao, S.V., S. Coca and K.K. Kalghatia (1977). Degradation of Phenolic compounds by the pathogenic fungus *Rhizoctonia Solani*. In "Physiology of Host Pathogen interaction" Ed. A. Mahadevan, Today and Tomorrow's Printers and Publishers. 432 – 483.
33. Sukhorukov, K.T. and N.A. Borodulina (1932). Nitrogen metabolism of alkaloidal Plants. *Bull. Acad. Sci*. 10: 1517.
34. Taubenhaus, J.J. and W.W. Ezekiel (1936). A rating of plants with reference to their relative resistance or susceptibility to *Phymatotrichum* root rot. *Texas. Agr. Exp. Sta. Bull.* 527: 1 – 52.
35. Wagner, H. (1977). Phenolic compounds in plants of pharmaceutical interests. In "Biochemistry of Plant Phenolics" ed. T. Swain, J.B.Harborne, C.F. Van Sumere, 589 – 616.
36. Wagner, H., S. Bladt and E.M. Zgainski (1984). Alkaloidal drugs. In "Plant Drug Analysis" Springer Verlag. New York, 55 – 60.
37. Weever, T. (1933). Die Pflanzen alkaloids phytochemisch and physiologisch betrachtet. *Res. Trav. Bot. Neerl*. 30: 336.
38. Weinges, K., P. Kloss, G. Trunzlov and E. Schuler (1971). *Plant Med*. **4**: 61.
39. Williams, R.T. (1959). "The pharmacology of plant phenolics". Academic Press, London. 14 – 15.