

ORIGINAL ARTICLE

A Comparative Assessment on Physicochemical Properties of Senna auriculata Leaves and Flowers of the Natural and Polluted Sources

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Received March 16, 2022

Background: The standardization of herbal medicines is an essential step in evaluating the quality of medicines based on drug concentration, physical and chemical standards. Due to the fact that the plant of *Senna auriculata* L. has a great medicinal potential with information about the compounds present in the various parts of the plant, it was considered important to study the preliminary physicochemical properties.

Results: Samples A & B were collected from natural habitats and Samples C & D were collected from polluted areas. Physicochemical properties of powdered plant material of *S. auriculata* include extractive value, ash value, loss on drying, and fluorescence behaviour were determined as per standard methods. The water-soluble extractive value in the leaves and flowers of samples A and B was greater than samples C and D. In terms of alcohol soluble extractive value, both samples A and B had a high percentage yield in their leaves and flowers, whereas samples C and D had a somewhat lower percentage yield. The leaves and flowers of samples A and B had lower moisture content percentages, whereas samples C and D had somewhat higher moisture content percentages.

Conclusions: The results of the physicochemical analysis suggest that samples A and B were far preferable to samples C and D in terms of quality. According to the findings of this study, collecting medicinal plants from their natural habitat is preferred since contaminated environments might impact the physicochemical properties of the plant material.

Key words: Senna auriculata, Physicochemical properties, Fluorescence behaviour, Natural sources, Polluted sources

Traditional medicine has been used for thousands of years with great contribution to human health by those skilled in the art, mainly as primary health care for a group of people and it has also retained its worldwide recognition (WHO, 2002). The traditional medical systems in India consist mainly of Ayurveda, Sidda and Unani. The systems are heavily dependent on medicinal plants. Due to its many biomedical uses, such as antibacterial. antifungal. cardioprotective. hepatoprotective, antidiabetic (Sujatha et al., 2018; Nagalingam et al., 2019; Pavunraj et al., 2019). The beneficial effects of medicinal plants and their maximum use can be achieved by other aspects, such as botany, ethnobotany, pharmacognosy, pharmacology. phytochemistry, biochemistry, biodiversity etc. Herbal drugs have been used around the world since the dawn of mankind and are of tremendous importance in international trade. The clinical, pharmaceutical and economic value of herbal medicines continues to grow, although it varies widely from country to country. From the traditional scientific study of remedies, many natural products have reached us and most of them are obtained from plants. Even if some of these crude pharmaceuticals have been gathered in less quantities for local use by local people and healers, several other raw medicines are harvested in larger numbers and marketed as raw materials for several herbal industries (Uniyal et al., 2006).

The basis of herbal products, which may contain powdered plant substances or extracts, remedies, and plant fatty oils. Extraction, fractionation, purification, concentration, and other physical or biological processes produce them. These are prepared by physical or biological processes. Standardization of herbal medicines is an important stage in determining the quality of medicines based on drug concentration, physical and chemical specifications. Each drug has its unique physicochemical characteristics that. like fingerprints, assist in understanding the drug and recognizing it from other drugs in the same category. With the commercialization of herbal medicine, it is vital to ensure the safety, uniqueness, and efficacy of medicinal plants and herbal products. S. auriculata bark,

flowers, leaves, roots, and unripe fruit combinations are used to make "Avarai Panchaga Choornam," which is used to treat blood glucose levels, urinary infections, conjunctivitis, and ophthalmia (Deshpande et al., 2013; Khader et al., 2017). Another beverage preparation known as "kalpa herbal tea" has S. auriculata (dried flowers) as its main component and is often used by those suffering from diabetes, urinary tract illnesses, and constipation (Thabrew et al., 2004). Many herbal products are made from leaves and flowers of S. auriculata, such as senna tablets, pickle, tea, idly powder, tonic, avaram poo sooranam, soup powder, diabetic food supplement. Due to the fact that the plant of Senna auriculata (L.) Roxb. has a great medicinal potential with information about the compounds present in the various parts of the plant, it was considered important to study the physicochemical properties of the plant.

MATERIAL AND METHODS

Plant material

In the present investigation we have selected one of the important medicinal plants, *Senna auriculata* (L.) Roxb. Tanner's cassia is a common name for such an evergreen plant. In the Ayurvedic and Siddha systems of medicine, the flower, buds, leaves, stem, root, and unripe fruit are recommended for treatment. It is commonly used to treat rheumatoid arthritis, conjunctivitis, and diabetes. The taxonomic classification is given in Table 1.

Collection and identification

S. auriculata leaves and flowers were taken from natural and contaminated sources in June 2021. For natural sources, two samples (A and B) were collected from unpolluted regions of site 1 (Pachaimalai hills) and site 2 (Kolli hills). Two samples (Samples C and D) were collected from the roadside in industrial regions. Site 3 is located on the Avur road, while Site 4 is located on the Samayapuram kariyamanickam road. The Botanical Survey of India (BSI), Southern Regional Centre, Coimbatore, validated the authenticity of the plant specimen (BSI/SRC/5/23/2021/Tech-166).

Physicochemical analysis

Physicochemical evaluation will help assess the

quality of the plant powder. Physicochemical properties of powdered plant material of *S. auriculata* include extractive value, (alcohol soluble extractive value, watersoluble extractive value), ash value (total ash value, water- soluble ash, acid insoluble ash), loss of drying, Fluorescence behavior were determined as per standard methods (WHO, 2000; Kokate *et al.*, 2002; Indian Pharmacopoeia, 2010).

Determination extractive value

The extractive value is the number of active elements extracted from plant material using solvents. It is generally calculated as the alcohol-soluble extractive value and the water-soluble extractive value.

Alcohol soluble extract value

Five grams of plant powder was macerated with 100 mL of alcohol in a conical flask. The flask was shaken repeatedly for the first 6 hours before being left to stand for 18 hours. The contents were quickly filtered through a dry filter paper. A 25ml filtrate sample was transferred to a tarred flat-bottomed dish and dried in 105° C water bath for 6 hours. The dried extract was cooled in desiccators for 30 minutes, weighed immediately and the value was used to calculate the extractive value and expressed in percentage.

Water-soluble extract value

Five grams of air-dried material were macerated in 100 ml of water for 24 hours in a reflux conical flask, shaken repeatedly for 6 hours, and allowed to stand for 18 hours. After rapidly filtering the solution, 25 ml of the filtrate was transferred to a tarred flat bottom dish and dried over a water bath. The extracts were subsequently dried in a hot air oven at 105 °C, and the residue was quantified. The percentage of extractive values for different solvents was calculated using the formula given below:

Extractive value (% w/w) = [(weight of residue ×100) / (25×weight of sample)] × 100

Determination ash value

Incineration of crude drugs results in the loss of organic material in the form of carbon dioxide and the formation of ash containing inorganic material such as carbonates, phosphates, sodium, potassium, calcium, and magnesium silicates. The ash value is a significant criterion for determining inorganic components and assessing the quality and purity of the crude drug in powdered form.

Total ash

The total ash is the total amount of material that remains after burning. This includes both physiological ash (obtained from the plant tissues) and nonphysiological ash (ashes are the remains of foreign objects attached to the surface of plants). Four grams of air-dried powdered plant material were precisely weighed and placed in a silicon crucible that had previously been burned and tarred. The material was spread equally and burnt, gradually increasing the temperature (>450 C) until it became white, confirming the lack of carbon. The substance was then weighed after cooling in a desiccator. The percentage of total ash concerning the air-dried powdered drugs was calculated using the expression given below:

Total ash (% w/w) = (weight of ash/weight of sample) × 100

Acid-insoluble ash

The acid-insoluble ash measures the amount of silica, especially sand and siliceous earth. In a crucible containing the total ash obtained by the above method, 25ml of 2N dilute HCl was added, then was covered with a watch-glass and boiled gently for 5 minutes. Rinse the watch glass with 5 ml of hot water and then pour this liquid into the crucible. The residue was collected on an ash-less filter paper, washed with hot water until the filtrate was neutral. Dry on a hot plate and burn under constant weight. Allow the residue to cool in a suitable desiccator for 30 minutes and then weigh. The percentage of acid-insoluble ash obtained in this way was calculated as follows:

Acid insoluble ash (% w/w) = (Weight of acidinsoluble ash/ Weight of sample) × 100

Water-soluble ash

Water-soluble ash is the difference between total ash and residue after treatment with water. The watersoluble ash is used to estimate the number of inorganic elements. The total ash (the ash obtained by the above method) was boiled with 25ml of water for 5 minutes. The insoluble matter was collected in a Gooch crucible and washed with hot water and heated at a low temperature not exceeding 450 °C for 15 min. Let the residue cool in a suitable desiccator for 30 minutes and then weigh. The weight of insoluble matter is subtracted from the weight of the ash taken for analysis. The difference in weight is the weight of the water-soluble matter in the ash. Use the following expression to calculate the percentage of water-soluble ash:

Water-soluble ash (% w/w) = (Weight of watersoluble ash/ Weight of sample) × 100

Determination loss on drying

For the determination of loss on drying, one gram of dried powdered leaf was accurately weighed in a tarred Petri dish. The powder was distributed as evenly as practicable, by gentle sidewise shaking. The dish was dried in an oven at 100 - 105 °C for 1 hour. It was cooled by desiccators and again weighed. The loss on drying was calculated regarding the amount of the dried powder taken.

Loss on drying (%w/w) =

<u>Difference between weight before and after drying</u> × 100 Weight of the sample before drying

Fluorescence analysis

The fluorescence analysis of plant powder may serve to evaluate the purity when the plant powders were treated with various acids and alkalis like HCl, H_2SO_4 , HNO_3 , HCl in methanol, and NaOH. The mixture was mixed well, allowed to stand for a few minutes, and filtered. The filtrate was observed under daylight as well as in UV light radiation at 365 nm and the colour exhibited was noted.

RESULTS AND DISCUSSION

The therapeutic efficacy of a medicinal plant is directly related to its quality. Adulterants in raw plant drugs and powdered drugs must be recognised as well. For this reason, several physiochemical properties can be used to standardise and maintain the 'quality assurance' of plant drugs. The following physicochemical evaluations were carried out quantitatively according to the procedures given in materials and methods: extractive value, ash value, loss on drying, and fluorescence behaviour.

The extractive values are useful for estimating the

chemical ingredients present in a crude drug and also for determining which constituents are soluble in a specific solvent. Extraction of any crude with a specific vields а solution containing solvent various phytoconstituents. The presence or absence of a certain component has a significant impact on the therapeutic value of the plant extract. The composition of these phytoconstituents in the specific solvent can provide preliminary information on the quality of a certain medicine sample (Tatiya et al., 2012). The water soluble extract values are critical in the evaluation of crude drugs. It can be used to denote poor quality, adulteration with any undesired material, or improper processing of the crude drug during the drying, storage etc. Table 2 reveals that the water soluble extractive value in the leaves and flowers of samples A and B was greater in percentage yield, but samples C and D had a slightly lower percentage yield. In terms of alcohol soluble extractive value, both samples A and B had a high percentage yield in their leaves and flowers, whereas samples C and D had a somewhat lower percentage yield. These findings highlighted that a slight decrease in percentage yield was mostly related to a lack of adequate nutrient supply. When the extractive values were compared, the alcohol soluble extractive value was greater than the water soluble extractive value. The presence of polar components such as sugar, acids, gum, and some inorganic compounds is indicated by the water-soluble extractive value. The presence of polar elements such as phenols, alkaloids, resins, steroids, glycosides, and flavonoids is indicated by the alcohol soluble extractive value (Jain et al., 2011; Kripa et al., 2016). The overall data demonstrated that alcohol is an option for extraction to produce the best results.

Ash values are significant quantitative standards for assessing the quality, identification, and purity of crude drugs, particularly in powdered form (Patnia *et al.*, 2012; Swamy and Mulla, 2010). The residue that remains after burning of plant material is known as ash value, and it simply indicates inorganic salts that are naturally found in crude medicine, adhering to it, or purposely added to it as a form of adulteration. The ash value of a crude drug is used to determine its quality and purity. It shows the presence of contaminants such as carbonate,

oxalate, and silicate. Three methods were used to calculate the ash value: total ash, acid-insoluble ash, and water-soluble ash. The total ash is the amount of ash that remains after ignition. This includes both 'physiological ash,' which is formed from plant tissue, and 'non-physiological ash,' which is the residue of extraneous matter adhering to the plant (Purohit et al., 2005). Acid insoluble ash is mostly composed of silica and indicates contamination by earthy material (Chanda, 2014). When acid insoluble ash is taken, a portion of the ash content is acid insoluble and so may be physiologically important as salt in the body. It also suggested that the plant was highly digestible when consumed (Ibrahim et al., 2010). Water soluble ash is the portion of total ash that is water soluble. The amount of inorganic component contained in drugs is estimated using water soluble ash. The ash values of S. auriculata leaves and flowers are presented in Table 3. These observations revealed the presence of silica, calcium oxalate, carbonates, silicates, and phosphates of sodium, calcium, potassium, and magnesium in S. auriculata ash.

A typical method for determining the moisture content of a powdered material is the loss on drying test. This might be triggered by water and any other volatile matter that can be driven out under certain conditions. The results of physicochemical investigation revealed that the percentage of moisture in natural and polluted samples varied. The leaves and flowers of samples A and B had lower moisture content percentages, whereas samples C and D had somewhat higher moisture content percentages (Table 4). This finding implies that the proportion of moisture in plants in contaminated environments is higher. The dry weight percent was seen to rise with an increase in the rate of pollution under greater stress environments. The amount of water or moisture contained in plant material determines the rate of deterioration. If the water content is high, fungal and bacterial growth can easily deteriorate the plant (Pandey and Tripathi 2014). Excess moisture can cause enzymatic activity to break down essential ingredients, which can increase the growth of yeast and fungi during storage (African Pharmacopoeia, 1986). The less in moisture level of drugs, the less probable bacterial, fungal, or yeast growth would arise while storage (Purwantiningsih *et al.*, 2011)

Fluorescence analysis is another critical pharmacognostic parameter. The fluorescence analysis of leaf powder of S. auriculata under day light as well as UV light are shown in Tables 5 & 6. The fluorescence behaviour of flower powder of *S. auriculata* from natural & polluted sources is noted in Tables 7 & 8. In daylight, several components are fluorescence in the visible range. Fluorescence is produced by ultraviolet light in many natural compounds that do not fluoresce conspicuously in daylight. If the chemical is not luminous on its own, it may usually be converted into fluorescent derivatives or metabolic byproducts by utilizing various reagents. As a result, crude pharmaceuticals are frequently assessed gualitatively in this manner, and it is an important criterion for crude drug pharmacognostic evaluation (Khandelwal, 2008; Zhao et al., 2011).

| Kingdom: | Plantae |
|-------------|-----------------|
| Division: | Magnoliophyta |
| Class: | Magnoliopsida |
| Sub Class: | Rosidae |
| Order: | Fabales |
| Family: | Fabaceae |
| Sub Family: | Caesalpiniaceae |
| Genus: | Senna |
| Species: | S. auriculata |

Table 1: Taxonomical classification

| | Extractive value (%w/w) | | | |
|---------|-------------------------|---------------|-----------------|---------------|
| Samples | Leav | /es | Flow | rers |
| | Alcohol soluble | Water-soluble | Alcohol soluble | Water-soluble |
| A | 18.6 | 12.5 | 16.4 | 13.1 |
| В | 13.2 | 10.7 | 11.2 | 10.4 |
| С | 07.5 | 04.9 | 13.6 | 08.5 |
| D | 10.4 | 07.3 | 10.7 | 05.1 |

Table 2 Extractive values of Senna auriculata leaves and flowers

A & B - Natural sources; C & D - Polluted sources. # Values are mean of three determinations

Table 3 Ash values of Senna auriculata leaves and flowers

| | Ash value (%w/w) | | | | | |
|---------|------------------|----------------|---------------|-----------|----------------|---------------|
| Samples | Leaves | | | Flowers | | |
| | Total ash | Acid-insoluble | Water-soluble | Total ash | Acid-insoluble | Water-soluble |
| A | 09.7 | 1.5 | 2.5 | 10.8 | 2.4 | 2.7 |
| В | 11.3 | 2.4 | 1.7 | 10.7 | 2.7 | 2.6 |
| С | 12.2 | 4.3 | 4.9 | 11.2 | 4.2 | 5.0 |
| D | 12.6 | 4.7 | 4.3 | 12.4 | 5.0 | 4.9 |

A & B - Natural sources; C & D - Polluted sources. # Values are mean of three determinations.

Table 4 L.O.D value of Senna auriculata leaves and flowers

| Constant | L.O.D value (%w/w) | | |
|----------|--------------------|---------|--|
| Samples | Leaves | Flowers | |
| A | 10.3 | 7.9 | |
| В | 10.7 | 8.6 | |
| С | 16.0 | 12.1 | |
| D | 11.2 | 12.7 | |

Note: - A & B - Natural sources; C & D - Polluted sources. # Values are mean of three determinations.

Table 5 Fluorescence behaviour of Senna auriculata leaves from natural sources

| | Observation under | | |
|---------------------------------------|-------------------|------------|--|
| Treatment | Day light | UV light | |
| Sample A | | | |
| Powder as such | Green | Green | |
| Powder+1N HCI | Brownish green | Black | |
| Powder+H ₂ SO ₄ | Dark green | Dark brown | |
| Powder+HNO ₃ | Brownish green | Dark green | |
| Powder+1N NaOH in methanol | Green | Green | |
| Sample B | | | |
| Powder as such | Greyish green | Green | |
| Powder+1N HCI | Brown | Black | |
| Powder+H ₂ SO ₄ | Dark green | Dark brown | |
| Powder+HNO ₃ | Dark green | Dark brown | |
| Powder+1N NaOH in methanol | Green | Green | |

| | Observation under | | |
|---------------------------------------|-------------------|---------------|--|
| Treatment | Day light | UV light | |
| | Sample C | | |
| Powder as such | Greyish green | Brown | |
| Powder+1N HCI | Brown | Black green | |
| Powder+H ₂ SO ₄ | Dark green | Brown | |
| Powder+HNO ₃ | Yellow green | Greyish green | |
| Powder+1N NaOH in methanol | Light Brown | Black green | |
| Sample D | | | |
| Powder as such | Brown | Brown | |
| Powder+1N HCI | Black | Dark green | |
| Powder+H ₂ SO ₄ | Brown | Dark brown | |
| Powder+HNO ₃ | Yellow green | Black green | |
| Powder+1N NaOH in methanol | Greyish green | Green | |

Table 6 Fluorescence behaviour of Senna auriculata leaves from polluted sources

Table 7 Fluorescence behaviour of Senna auriculata flowers from natural sources

| | Observation under | | |
|---------------------------------------|-------------------|------------------|--|
| Treatment | Day light | UV light | |
| | Sample A | | |
| Powder as such | Yellowish green | Yellowish green | |
| Powder+1N HCI | Light orange | Brown | |
| Powder+H ₂ SO ₄ | Yellowish orange | Dark brown | |
| Powder+HNO ₃ | Orange | Blackish orange | |
| Powder+1N NaOH in methanol | Yellow | Yellow | |
| Sample B | | | |
| Powder as such | Yellowish orange | Yellowish orange | |
| Powder+1N HCI | Brown | Dark brown | |
| Powder+H ₂ SO ₄ | Light orange | Black | |
| Powder+HNO ₃ | Orange | Brown | |
| Powder+1N NaOH in methanol | Yellow | Yellow | |

Table 8 Fluorescence behaviour of Senna auriculata flowers from polluted sources

| | Observation under | | |
|---------------------------------------|-------------------|-----------------|--|
| Treatment | Day light | UV light | |
| | Sample C | | |
| Powder as such | Yellowish brown | Brown | |
| Powder+1N HCI | Light orange | Blackish orange | |
| Powder+H ₂ SO ₄ | Yellowish orange | Brown | |
| Powder+HNO ₃ | Orange | Black | |
| Powder+1N NaOH in methanol | Yellow | Dark brown | |
| Sample D | | | |
| Powder as such | Yellowish orange | Brown | |
| Powder+1N HCl | Orange | Dark brown | |
| Powder+H ₂ SO ₄ | Yellowish orange | Blackish orange | |
| Powder+HNO ₃ | Orange | Black | |
| Powder+1N NaOH in methanol | Brown | Dark brown | |

CONCLUSION

All the above data suggests that samples A and B were far preferable than samples C and D in terms of quality. This is due to the fact that samples A and B

were collected from relatively pollution-free natural habitats, whereas samples C and D were collected from polluted environments. According to the findings of this study, collecting medicinal plants from their natural habitat is preferred since contaminated environments might impact the physicochemical properties of the plant material.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

REFERENCES

- African Pharmacopoeia. (1986) General Methods of Analysis Pharmacopoeia, 11(1st Ed.). pp 121-208.
- Chanda S. (2014) Importance of pharmacognostic study of medicinal plants: an overview. *J. pharmacogn. phytochem.* 2(5): 69-73.
- Deshpande S., Kewatkar S., and Paithankar V. (2013) Antimicrobial activity of saponins rich fraction of *Cassia auriculata* Linn against various microbial strains. *Int. Curr. Pharm. J.* 2(4): 85–87.
- Ibrahim J., Ajaegbu V.C., and Egharevba H.O. (2010) Pharmacognostic and Phytochemical Analysis of Commelina benghalensis L. Ethnobot. leafl. 14(5): 610–615.
- Indian Pharmacopoeia. (2010) Government of India Ministry of Health & Family Welfare, Ghaziabad, Vol. III.
- Jain S., Sharma C., Khatri P., Jain A., and Vaidya A. (2011) Pharmacognostic and phyto chemical investigations of the leaves of *Zizyphus xylopyrus* (Retz) willd. *Int. J. Pharm. Pharm. Sci.* 3(2): 122-5.
- Khader S.Z.A, Ahmed S.S.Z., Balasubramanian S.K., Arunachalam T.K., Kannappan G., Mahboob M.R., Ponnusamy P., and Ramesh K. (2017) Modulatory effect of dianthrone rich alcoholic flower extract of *Cassia auriculata* L. on experimental diabetes. *Integr. Med. Res.* 6 (2): 131-140.
- Khandelwal K. (2008) Practical pharmacognosy. Pragati Books Pvt. Ltd, Nagpur.
- Kokate C.K., Purohit A.P., and Gokhale S.B. (2002) Text Book of Pharmacognosy, 18th Ed. Pune: Nirali Prakashan.
- Kripa K.G., Sangeetha R., and Chamundeeswari D. (2016) Pharmacognostical and physicochemical evaluation of the plant *Leucas aspera*. Asian J. Pharm. Clin. Res. 9(2): 263-8.

- Nagalingam M., Vikramathithan M., Dhanesh G.A., and Rajeshkumar S. (2019) Evaluation of herbal and chemical-based mouthwash against oral pathogens. *Drug Invent. Today.* 11(1): 147-151.
- Pandey A., and Tripathi A. (2014) Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. J. pharmacogn. phytochem. 2(5): 115-119.
- Patnia S., Saha A.N., Meena H., Pandey H.K., and Manchanda A. (2012) Physicochemical, phytochemical and elemental analysis of stem bark and roots of *Berberis asiatica*. *Adv. App. Sci. Res.* 3(6): 3624-3628.
- Pavunraj M., Rajeshkumar S., Bhuvana L., and Babujanarthanam R. (2019) Preparation of *Cassia auriculata* plant extracts using different solvents and its antibacterial and antifungal activity against clinical pathogens. *Drug Invent. Today.* 11(1): 142-146.
- Purohit A.P., Kokate C.K., and Gokhale S.B. (2005) Pharmacognosy 13th Edition, Nirali prakashan India. pp 256-259.
- Purwantiningsih, Purwantin I., and Djoko S. (2011) Identification of standard parameters of kepel leaves [*Stelechocarpus Burahol* (Bl.) Hook. F. and Th.] And the extract as raw material for antihyperuricemic medicaments. *Asian J. Pharm. Clin. Res.* 4(1): 149-53.
- Sujatha J., Asokan S., and Rajeshkumar S. (2018) Phytochemical analysis and antioxidant activity of chloroform extract of *Cassis alata*. *Res J Pharm Technol.* 11(2): 439-444.
- Swamy P., and Mulla S.K. (2010) Preliminary pharmacognostical and phytochemical evaluation of *Portulaca quadrifida* Linn". *Int. J. Pharmtech Res.* 2(3): 1699-1702.
- Tatiya A., Surana S., Bhavsar S., Patil D., and Patil Y.
 (2012) Pharmacognostic and preliminary phytochemical investigation of *Eulophia herbacea* Lindl. Tubers (Orchidaceae). Asian Pac. J. Trop. Dis. 2(Suppl 1): S50 S55
- Thabrew M.I., Munasinghe T.M.J., Senarath S., and Yapa R.M.S.C. (2004) Effects of *Cassia auriculata*

and *Cardiospermum halicacabum* teas on the steady state blood levels of theophylline in rats. *Drug Metabol Drug Interact.* 20(4): 263-272.

- Uniyal S.K., Singh K.N., Jamwal P., and Lal B. (2006)
 Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalaya. *J. Ethnobiol. Ethnomedicine.* 2(1): 1–8.
- WHO, (2000) World Health Organization: General guidelines for methodologies on research and

evaluation of traditional medicine, Geneva.

- WHO, (2002) World Health Organization: Traditional Medicine Strategy Report.
- Zhao Z., Liang Z., and Guo P. (2011) Macroscopic identification of Chinese medicinal materials: traditional experiences and modern understanding. *J. Ethnopharmacol.* 134(3): 556-564.